

**Atf için:** Törün B, Bıyık H.H, Poyrazoğlu E. 2022. Morphological and Molecular Identification of Biofilm Forming Fungi from Fish Farms and Fish Benches in Aydın Province. İğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi. 12(1): 115-124.

**To Cite:** Törün B, Bıyık H.H, Poyrazoğlu E. 2022. Morphological and Molecular Identification of Biofilm Forming Fungi from Fish Farms and Fish Benches in Aydın Province. Journal of the Institute of Science and Technology. 12(1): 115-124.

**Morphological and Molecular Identification of Biofilm Forming Fungi from Fish Farms and Fish Benches in Aydın Province**

Bahadır TÖRÜN<sup>1\*</sup>, H. Halil BIYIK<sup>1</sup>, Esin POYRAZOĞLU<sup>1</sup>

**ABSTRACT:** Biofilm formation is one of the major problems in many industries. The marine environment is rich in nutrients for microbial growth. It also makes it easier for microorganisms to form biofilms. This study aims to identify biofilm-forming fungi in the marine environment and assess their ability to form biofilm. We identified biofilm-forming fungi species using morphological and molecular methods. ITS regions were used for molecular identification. The qualitative assessment of biofilms was carried out using the Petri dish method, and quantitative measurements of biofilms were carried out using the microplate method. We identified 69 isolates; 37 were yeast, and 32 of them were fungi. Only ten of them were found to have biofilm. Most of them were adherents, and only two of them were strong adherents. Biofilms can have positive or negative effects on fish health. Identifying biofilm-forming agents will help us identify the nature of the effect. Microorganisms that have positive effects can be used as biocontrol agents.

**Keywords:** Biofilm, Fungi, ITS, Seabass, Seabream

<sup>1</sup>Bahadır TÖRÜN ([Orcid ID: 0000-0001-5142-1882](https://orcid.org/0000-0001-5142-1882)), H. Halil BIYIK ([Orcid ID: 0000-0003-0258-054X](https://orcid.org/0000-0003-0258-054X)), Esin POYRAZOĞLU ([Orcid ID: 0000-0002-3921-5362](https://orcid.org/0000-0002-3921-5362)) Aydın Adnan Menderes Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Aydın, Türkiye

\*Sorumlu Yazar/Corresponding Author: Bahadır TÖRÜN, e-mail: bahadrtrn@gmail.com

## INTRODUCTION

Most aquatic fungi do not cause disease in fish. In addition, most pathogenic fungi are free-living and pose no threat. However, biofilms, bacterial or fungal, provide a suitable and protective environment for potential pathogens (Turgay et al, 2019). Most of the aquatic fungi get their nutrients by decomposing organic matter (Chouldhury et al, 2013). They mostly cause secondary infections in immunocompromised fish (Roberts, 2012). *Oomycetes* are most common group of fungal pathogens (Chouldhury et al, 2013). Genus *Saprolegnia* is the most common fungal pathogen of fishes both in fresh and saltwater (Gaikowski et al, 2003; Dinçtürk et al, 2018).

A biofilm is a community of microorganisms surrounded by an extracellular polymeric substance attached to each other and to the surface (Vert et al, 2012). Biofilm communities can contain one or more species. If a biofilm contains more than one species, they are called polymicrobial biofilms (Broden et al, 2005). The biofilm protects sterilizer agents, antimicrobials, environmental factors, and other biological agents such as phages (Connel et al, 2010).

Because it contains excessive feed and fish excrement, a controlled water environment is suitable for microbial growth. In addition, surfaces such as tanks and pipes are ideal for biofilm formation. There are several studies on biofilm formation in aquaculture (Kuranasagar et al, 1996; You et al, 2007; Wietz et al, 2009; Pandey et al, 2014; Santhakumari et al, 2016; Cai and Arias, 2017; Arankumar et al, 2020). However, most of the research focuses on bacterial biofilms. Studies also show that fungal species can also produce biofilms (Imamura et al, 2008; Costa-Orlandi et al, 2014; Sardi et al, 2015; Gonzales-Ramirez et al, 2016). Some research has focused on the beneficial effects / use of biofilms in aquaculture (Panigrahi and Azad, 2007; Wesselling et al, 2015; Barnharst et al, 2015).

This study aims to identify biofilm-forming fungi in aquatic environments. A better understanding of these organisms can provide us with useful information for solving some of the problems in aquaculture. Our research focuses on cultured organisms because we want to conduct in vitro biofilm formation tests with each organism.

## MATERIALS AND METHODS

### Materials

Samples were taken from fish farm hatcheries and fish stalls in Aydın province in 2017. Samples were taken under aseptic conditions from skin and gills of fishes, tank and pipe surfaces of sea bass (*Dicentrarchus labrax*), and sea bream (*Sparus aurata*) cultures and stalls.

### Isolation and identification

Samples were taken with a sterile swab and stored in 0,9 % saline water until arrival to the laboratory. Series of dilutions ranging from  $10^0$ - $10^{-4}$  were prepared (Koch, 1883). Rose-Bengal Chloramphenicol Agar was used as the first culture medium. Isolation of the individual organisms was performed on Potato Dextrose Agar. Cultures were incubated at 27 °C for 2 to 7 days. Colonies were examined in size (mm), shape, color, exudation, and pigmentation. Their microscopic properties were examined under stereomicroscope and light microscope. Fungi were identified at the genus level using Barnett (2003) "Illustrated genera of imperfect fungi". For yeasts, only molecular identification was performed.

Genomic DNA isolation was made according to the 2X CTAB DNA isolation method (Doyle and Doyle, 1987). Molecular identification of the isolates performed with rDNA ITS regions. For yeasts ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3') primers, and fungi ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT GCT

TAT TGA TAT GC-3') were used (Op de Beeck, 2014). The amplification protocol consisted of initial denaturation at 95 °C for 3 min, followed by 35 cycles with denaturation 94 °C for 30 s, annealing 58 °C for 30 s, and extension 72 °C for 60 s. The reaction was completed with a 72 °C 10 min final extension step. Fermantas 2X PCR Master Mix was used as PCR components. PCR products were sent to sequencing (GATC, Germany). Obtained ITS sequences were aligned with the ones in the GenBank using BLASTn software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic analysis was made according to maximum likelihood with MEGA X software.

### Biofilm tests

Two methods were used for biofilm formation: petri method for qualitative and microplate method for quantitative.

For qualitative tests isolates were inoculated on Sabouraud Dextrose Agar (SDA) for 24 hours. Ten milliliters of Sabouraud dextrose broth (SDB) was poured into Petri dishes, and the fungal colony was transferred from SDA to SDB. Incubated for 24 hours at 37 °C. After incubation, the broth from petri dishes was discarded and washed twice with 5 ml distilled water. Petri dishes were then stained with 2% safranin. After one minute, the Petri dishes were washed with 5 ml of distilled water to remove excess dye and allowed to dry at room temperature. Petri dishes were examined under a microscope. The presence of fungal cells and hyphae covering all areas was accepted as positive for the biofilm, while sparse areas of fungus or the absence of fungi were considered negative (Costa-Orlandi et al, 2014).

For quantitative tests biofilm formation was measured according to Christensen et al. (1985) with modifications. Fresh strains with 0.5 McFarland turbidity were obtained and suspended with 0.9% (w / v) Saline. Then, 1 ml of the prepared suspensions were transferred to 9 ml tubes containing SDB and incubated at 37 °C for 24 hours. After incubation, 150 µL of suspension was taken from each tube and transferred in triplicate to a 96-well polystyrene microtiter plate. Plates were incubated at 37 °C for 48 hours. After incubation, the liquid medium was discarded, the wells were washed 3 times with distilled water and allowed to dry. Then 150 µl of crystal violet solution (0.5% v / v) was dispensed into the wells and incubated at room temperature for 45 minutes. After incubation, the wells were washed 3 more times with distilled water and dried. 150 µl ethanol: acetic acid (95: 5) was added to the wells and incubated for 10 minutes. Then 100 µl of liquid from each well was transferred to a new microtiter plate and the absorbance was determined at 570 nm on a spectrophotometer. Sterile distilled water was used as negative control and *Pseudomonas aeruginosa* PAO1 strain was used as positive control. Strains with optical density values above 0.240 were considered strong adhesion, strains with 0.120-0.240 were considered adhesion, and strains 0.120 and below were considered negative.

## RESULTS AND DISCUSSION

### Isolation and identification

We isolated a total of 69 fungal species. Thirty-seven of these are yeasts and 32 of them are fungi (Table 1 and 2). Among these 69 isolates, 17 different genera were found.

**Table 1.** List of fungal species

Sample Name	Species	% Similarity
K4	<i>Penicillium glabrum</i>	98%
K5	<i>Aspergillus flavus</i>	95%
K7	<i>Cladosporium cladosporioides</i>	94%
K17	<i>Penicillium urticae</i>	99%
K19	<i>Aspergillus cristatus</i>	96%
K21	<i>Cladosporium cladosporioides</i> 2	97%
K22	<i>Trichosporon lactis</i>	96%

**Table 1.** List of fungal species (continue)

K23	<i>Aspergillus amstelodami</i>	99%
K24	<i>Apiotrichum montevidense</i>	95%
K25	<i>Pyrenochaeta quercina</i>	99%
K26	<i>Penicillium chrysogenum</i>	98%
K27	<i>Cutaneotrichosporon jirovecii</i>	99%
K29	<i>Trichosporon lactis</i>	97%
K30	<i>Cladosporium macrocarpum</i>	98%
K31	<i>Penicillium dravuni</i>	98%
K32	<i>Ramichloridium apiculatum</i>	98%
K33	<i>Cladosporium cladosporioides 3</i>	97%
K34	<i>Trichosporon lactis 2</i>	99%
K35	<i>Trichosporon sp.</i>	98%
K36	<i>Penicillium brevicompactum</i>	97%
K37	<i>Trichosporon lactis 2</i>	98%
K38	<i>Alternaria tenuissima</i>	93%
K39	<i>Alternaria tenuissima 2</i>	98%
K40	<i>Cladosporium cladosporioides 4</i>	99%
K41	<i>Cladosporium velox</i>	99%
K45	<i>Alternaria tenuissima 3</i>	99%
K48	<i>Trichoderma citrinoviride</i>	99%
K49	<i>Penicillium spinulosum</i>	100%
K50	<i>Penicillium antarcticum</i>	96%
K51	<i>Aspergillus amstelodami</i>	99%
L8	<i>Cladosporium macrocarpum</i>	97%
L9	<i>Trichoderma citrinoviride 2</i>	99%

**Table 2.** List of yeast species

Sample Name	Species	% Similarity
L1	<i>Rhodotorula babjevae</i>	99%
L2	<i>Rhodotorula babjevae 2</i>	99%
L3	<i>Rhodosporidium sphaerocarpum</i>	97%
L5	<i>Candida zeylanoides</i>	98%
L6	<i>Candida zeylanoides 2</i>	99%
L7	<i>Candida zeylanoides 3</i>	99%
L11	<i>Hanseniaspora uvarum</i>	98%
L12	<i>Hanseniaspora uvarum 2</i>	87%
L14	<i>Rhodotorula diobovata</i>	99%
L15	<i>Rhodosporidium sphaerocarpum</i>	97%
L16	<i>Cystofilobasidium infirmominiatum</i>	99%
L17	<i>Rhodotorula sphaerocarpa</i>	99%
L19	<i>Candida zeylanoides 4</i>	99%
L20	<i>Debaryomyces sp.</i>	98%
L21	<i>Rhodotorula mucilaginoso</i>	97%
L22	<i>Candida zeylanoides strain 5</i>	97%
L23	<i>Rhodotorula mucilaginoso 2</i>	99%
L24	<i>Debaryomyces hansenii</i>	99%
L25	<i>Candida galli 1</i>	98%
L27	<i>Candida galli 2</i>	96%
L28	<i>Candida zeylanoides 5</i>	98%
L30	<i>Candida atlantica</i>	97%
L33	<i>Rhodotorula mucilaginoso 3</i>	98%
L34	<i>Candida zeylanoides 6</i>	99%
L35	<i>Candida atlantica 2</i>	98%

**Table 2.** List of yeast species (continue)

L36	<i>Rhodotorula kratochvilovae</i>	97%
L37	<i>Rhodospordium sp.</i>	97%
L38	<i>Debaryomyces hansenii 2</i>	99%
L39	<i>Debaryomyces hansenii 3</i>	99%
L40	<i>Debaryomyces hansenii 4</i>	99%
L41	<i>Debaryomyces hansenii 5</i>	99%
L42	<i>Yarrowia galli</i>	98%
L45	<i>Candida zeylanoides 7</i>	99%
K3	<i>Candida galli 3</i>	99%
K16	<i>Candida galli 4</i>	99%
K18	<i>Candida galli 5</i>	99%
K47	<i>Exophiala xenobiotica</i>	99%

Phylogenetic analysis was made with MEGA X software according to the maximum likelihood method. Phylogenetic tree obtained for fungi and yeast species were shown in Figure 1 and 2.

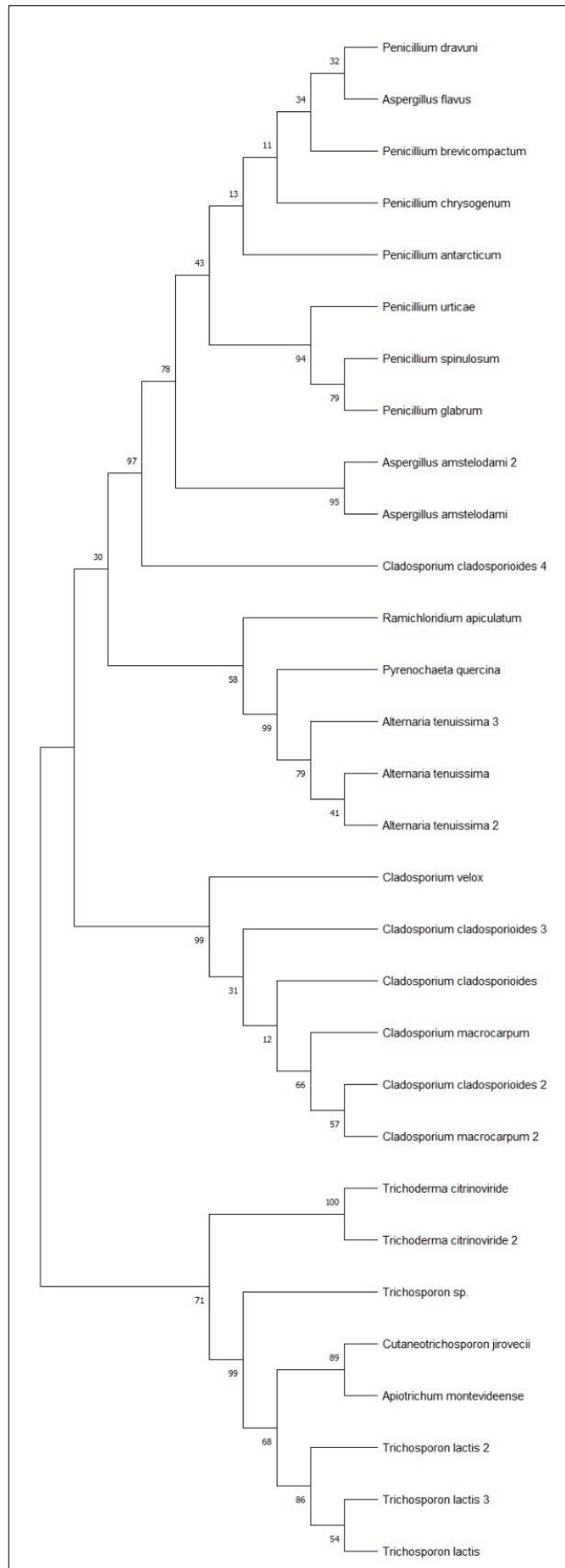
In our study, we found only 14,5 % of the fungal species are forming biofilms. Although this number seems low, combined with other biotic and abiotic factors, they might become important for fish and human health. A previous study about the fungal diversity of marine biofilms in the Gulf of Mexico has similar findings with our study with regards to fungal diversity (Salamone et al, 2016). All of the fungi we isolated belong to facultative marine genera: "those from freshwater or terrestrial milieus able to grow in the marine environment" (Kohlmayer, 1974). None of our isolates are considered fish pathogens but *Candida* species are known human pathogens (Caldarone and Clancy, 2011). Some of the species were also reported as plant pathogens (Luque et al, 2000; Miao and Qian, 2005). These human and plant pathogens probably came from the staff. There are aquaculture engineers but most of the staff are seasonal workers from surrounding villages. Most of them are not careful with their personal hygiene and almost all of them works in their farms. Miao and Qian (2005) studied the antagonistic antimicrobial activity of marine fungi and bacteria from marine biofilms. They found most of the fungal isolates inhibited at least one bacteria. We also found *Alternaria*, *Trichoderma* and *Cladosporium* genera which they found antibacterial properties. Siqueira and Lima (2013) showed *Aspergillus*, *Alternaria*, *Botrytis*, *Cladosporium* and *Penicilium* genera are capable of forming biofilms in aquatic environments (Siquiera and Lima, 2013). We also showed *Alternaria* and *Aspergillus* genera can form biofilms.

Most of the species are from fish stalls. In farms fish tanks are cleaned daily. This is the possible reason of low numbers of species from fish farm. Also in fish farms same people does the maintenance of tanks while fish stalls were visited by a large number of people daily. *Candida* species came from both fish farm and fish stalls. Also *Aspergillus* and *Penicilium* species were both present in fish farms and fish stalls. *Exophiala xenobiotica*, *Yarrowia galli* were found in fish farm and *Cystofilobasidium infirmominiatum*, *Hanseniaspora uvarum* were found in fish stalls.

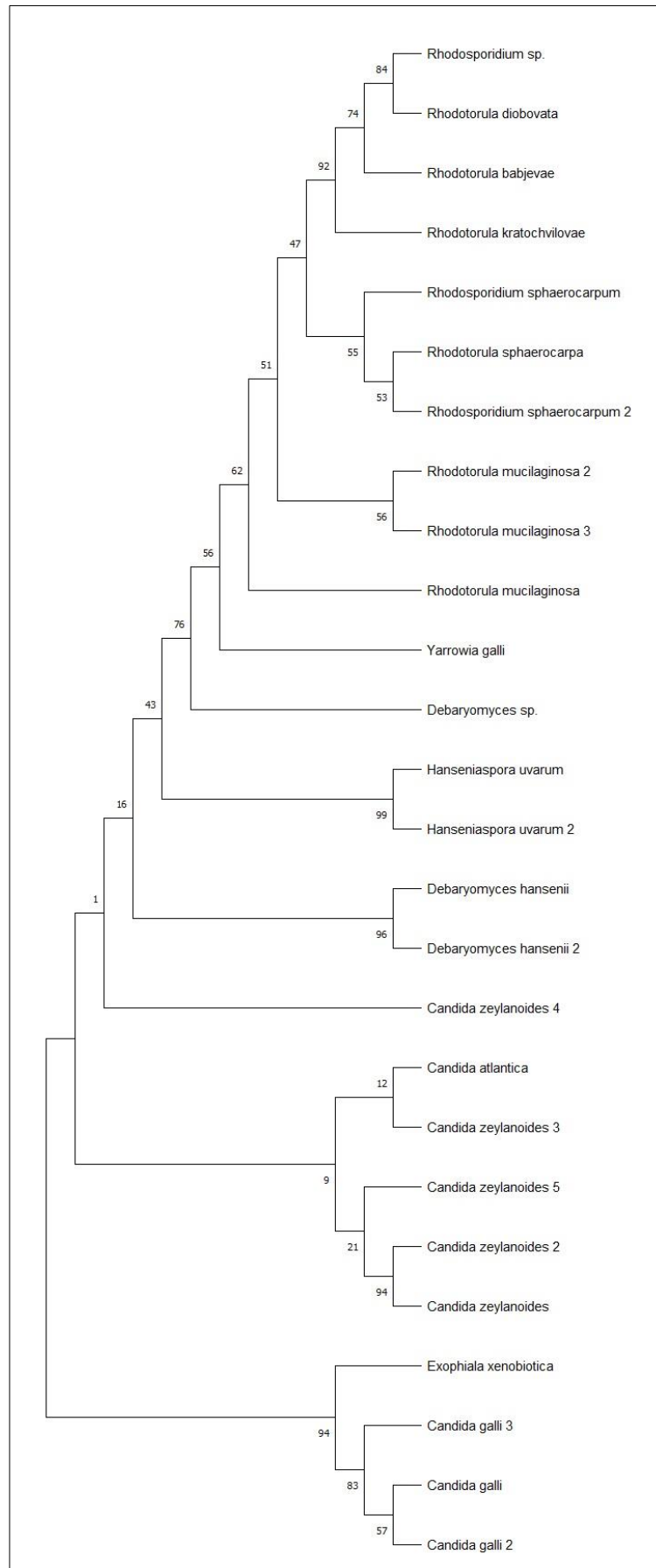
### Biofilm tests

We found only 10 isolates biofilm positive among 69 isolates and 17 different genera. Biofilm results were given in Table 3.

The majority of the species are moderate adherent. Two of the species are strong adherent and two of them are weak adherent. This will affect the removal of biofilms from surfaces. Strong adherent species will be harder to remove from the surfaces.



**Figure.1.** Phylogenetic tree of fungi species. Evolutionary analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model. Evolutionary analyses were conducted in MEGA X.



**Figure 2.** Phylogenetic tree of yeast species. Evolutionary analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. Evolutionary analyses were conducted in MEGA X.

**Table 3.** Biofilm forming isolates and their adherence

No	Name	Adherence
1	<i>Aspergillus flavus</i>	Adherent
2	<i>Aspergillus amstelodami</i>	Adherent
3	<i>Alternaria tenuissima</i>	Weak adherent
4	<i>Trichosporon lactis</i>	Weak adherent
5	<i>Candida galli</i>	Strong adherent
6	<i>Candida zeylanoides</i>	Strong adherent
7	<i>Exophiala xenobiotica</i>	Adherent
8	<i>Rhodotorula kratochvilovae</i>	Adheran
9	<i>Rhodosporidium sphaerocarpum</i>	Adherent
10	<i>Debaryomyces hansenii</i>	Adherent

From these ten biofilm forming species only one belongs to fish farm, *Exophiala xenobiotica*, other species comes from fish stalls.

There is a complicated relationship between microorganisms and their environment. This is an intriguing subject to study. Biofilms are one of the most sophisticated structures of the microbial world. This study identifies fungal communities of marine environments and tests their biofilm capabilities. We provide initial data for some biofilm-forming fungi in marine environments.

A metagenomic study can give us the list of all organisms but in this study, we focused on culturable species because we wanted to simulate biofilm formation *in-vitro*. Our study only assesses the biofilm-forming capabilities of cultured species. Further studies must be done for uncultured species and their roles in biofilms.

## CONCLUSION

There is a complex relationship between microorganisms and their environment. Biofilms are one of the most complex structures in the microbial world. This study identifies fungal communities in the marine environment and tests their biofilm capacity. We provide baseline data for some biofilm-forming fungi in the marine environment.

Although there are not many fungal pathogens reported for fish, their presence can affect pathogens. This effect can be positive or negative depending on the situation. By forming biofilms fungi can accomodate and protect pathogens. This could emerge disease and prolong treatments causing time loss and financial loss. Prolonged treatments can result in multidrug resistant strains of pathogens. If we know the type and the strength of the biofilm we can take necessary precautions to avoid unwanted situations. Further studies are needed to identify which biofilm communities have positive or negative effects. If positive biofilm communities are stimulated, they can be used as biological control agents.

## ACKNOWLEDGEMENTS

This study was conducted in Aydın Adnan Menderes University, Faculty of Science and Literature, Biology Department, Microbiology Laboratory with the support of the Scientific Research Department (Project No: FEF-17025). We would like to thank M.Sc. Mehmet Ali Yörükce and M.Sc. Fatma Yaman for their contributions.

## Conflict of Interest

The article authors declare that there is no conflict of interest between them.

## Author's Contributions

The authors declare that they have contributed equally to the article.



## REFERENCES

- Arunkumar M, Lewis-Oscar F, Thajuddin N, Pugazhendhi A, Nithya C, 2020. *In vitro* and *in vivo* biofilm forming *Vibrio* sp: A significant threat in aquaculture. *Process Biochemistry* 94:213-223.
- Barnett H.L, 2003. Illustrated genera of Imperfect fungi. Burgess Publishing Company Minneapolis-USA.
- Barnharst T, Rajendran A, Hu B, 2018. Bioremediation of synthetic intensive aquaculture wastewater by a novel feed-grade composite biofilm. *Int Biodeterior and Biodegradation* 126:131–142.
- Brogden K. A, Guthmiller J. M, Taylor C. E, 2005. Human polymicrobial infections. *The Lancet* 365(9455):253-255.
- Cai W, Arias, CR, 2017. Biofilm formation on aquaculture substrates by selected bacterial fish pathogens. *Journal of Aquatic Animal Health*, 29(2): 95–104.
- Calderone RA, Clancy C J, 2011. *Candida* and candidiasis. American Society for Microbiology Press Baltimore-USA.
- Choudhury GT, Singh SK, Parhi J, Barman D, Das B. 2014. Common Fungal Diseases of Fish: A Review. *Environment and Ecology*. 32. 450-456.
- Christensen GD, Simpson WA, Younger JA, Baddour LM, Barrett FF, Melton DM et al, 1985. Adherence of coagulase negative staphylococci to plastic tissue cultures: A quantitative model for the adherence of staphylococci to medical devices. *Journal of Clinical Microbiology*, 22(6): 906-1006.
- Connell JL, Wessel AK, Parsek MR, Ellington AD, Whiteley M, Shear JB, 2010. Probing prokaryotic social behaviors with bacterial “lobster traps”. *MBio* 1(4):1-8.
- Costa-Orlandi CB, Sardi JC, Santos CT, Fusco-Almeida AM, 2014. In vitro characterization of *Trichophyton rubrum* and *T. mentagrophytes* biofilms. *Biofouling* 30(6):719-727.
- Dinçtürk E., Tanrıkul T.T., Türk Çulha S. 2018. Fungal and Bacterial Co-Infection of Sea Bass (*Dicentrarchus labrax*, Linnaeus 1758) in a Recirculating Aquaculture System: *Saprolegnia parasitica* and *Aeromonas hydrophila*. *Aquatic Sciences and Engineering*, 33(3): 67-71
- Doyle J.J, Doyle J. L, 1987. Isolation of Plant DNA From Fresh Tissue. *Focus*, 12, 13-15.
- Gaikowski M. P., Rach J. J., Drobish M., Hamilton J., Harder T., Lee L. A., Moen C., Moore A. 2003. Efficacy of hydrogen peroxide in controlling mortality associated with saprolegniasis on walleye, white sucker, and paddlefish eggs. *North American Journal of Aquaculture* 65(4), 349-355.
- Gonzalez-Ramirez AI, Ramirez-Granillo A, Medina-Canales MG, Rodriguez-Tovar AV, 2016. Analysis and description of the stages of *Aspergillus fumigatus* biofilm formation using scanning electron microscopy. *BMC Microbiology*, 16(1): 243.
- Imamura Y, Chandra J, Mukherjee PK, Lattif AA, Szczotka-Flynn LB, Pearlman E, Lass JH, O'Donnell K, GMA, 2008. *Fusarium* and *Candida albicans* biofilms on soft contact lenses: Model development, influence of lens type, and susceptibility to lens care solutions. *AAC*, 51(1): 171-182.
- Karunasagar I, Otta S. K, Karunasagar I, 1996. Biofilm formation by *Vibrio harveyi* on surfaces. *Aquaculture* 140(3):241–245.
- Koch R, 1999. Über die neuen Untersuchungsmethoden zum Nachweis der Mikrokosmen in Boden, Luft und Wasser. Vortrag auf dem XI. Deutschen Ärztetagen Berlin. Vereinsblatt für Deutschland, Kommissions-Verlag von F. C. W. Vogel, Leipzig 137:274-284.
- Kohlmeyer J, 1974. On the definition and taxonomy of higher marine fungi. *Veroff. Inst. Meerforsh. Bremerhaven. Suppl*, 5: 263–286.
- Luque J, Parladé J, Pera J, 2000. Pathogenicity of fungi isolated from *Quercus suber* in Catalonia (NE Spain). *Forest Pathology* 30(5): 247-263.
- Miao L, Qian PY, 2005. Antagonistic antimicrobial activity of marine fungi and bacteria isolated from marine biofilm and seawaters of Hong Kong. *Aquatic Microbial Ecology* 38: 231–238.
- Op De Beeck M, Lievens B, Busschaert P, Declerck S, Vangronsveld J, et al, 2014. Comparison and Validation of Some ITS Primer Pairs Useful for Fungal Metabarcoding Studies. *PlosOne*. 9(6): e97629.
- Pandey P. K, Bharti, V, Kumar K, 2014. Biofilm in aquaculture production. *African Journal of Microbiological Research* 8(13):1434–1443.
- Panigrahi A, Azad, IS, 2007. Microbial intervention for better fish health in aquaculture: the Indian scenario. *Fish Physiology and Biochemistry* 33(4):429-440.
- Roberts RJ. 2012. *Fish pathology*. 4th ed. Wiley- Blackwell, USA.
- Romano C, Valenti L, Miracco C, Alessandrini C, Paccagnini E, Faggi E, Difonzo E. M, 1997. Two cases of cutaneous phaeohyphomycosis by *Alternaria alternata* and *Alternaria tenuissima*. *Mycopathologia*, 137(2):65–74.

- Salamone A. L, Robicheau B. M, Walker A. K, 2016. Fungal diversity of marine biofilms on artificial reefs in the north-central Gulf of Mexico. *Botanica Marina* 59(5): 291–305.
- Santhakumari S, Kannappan A, Pandian S. K, Thajuddin N, Rajendran R. B, Ravi A. V, 2016. Inhibitory effect of marine cyanobacterial extract on biofilm formation and virulence factor production of bacterial pathogens causing vibriosis in aquaculture. *Journal of Applied Phycology* 28(1):313-324.
- Sardi JC, Pitangui NS, Voltan AR, Braz JD, Machado MP, Fusco Almeida AM, 2015. In vitro *Paracoccidioides brasiliensis* biofilm and gene expression of adhesins and hydrolytic enzymes. *Virulence*, 6(6):642-651
- Siqueira VM, Lima N, 2013. Biofilm Formation by Filamentous Fungi Recovered from a Water System. *Journal of Mycology* Article ID 152941.
- Turgay E, Stenium T, Colguhoun D, Karataş S, 2019. Environmental biofilm communities associated with early-stage common dentex (*Dentex dentex*) culture. *Journal of Applied Microbiology* 126(4):1032-1043.
- Vert M, Doi Y, Hellwich K. H, Hess M, Hodge P, Kubisa P, et al, 2012. Terminology for biorelated polymers and applications (IUPAC Recommendations). *Pure Applied Chemistry* 84(2):377-410.
- Wesseling W, Wittka S, Kroll S, Soltmann C, Kegler P, Kunzmann A, Lohmeyer M, 2015. Functionalised ceramic spawning tiles with probiotic *Pseudoalteromonas* biofilms designed for clownfish aquaculture *Aquaculture* 446:57–66.
- Wietz M, Hall M. R, Høj L, 2009. Effects of seawater ozonation on biofilm development in aquaculture tanks. *Systematic and Applied Microbiology* 32(4):266–277.
- You J, Xue X, Cao L, Lu X, Wang J, Zhang L, Zhou S, 2007. Inhibition of *Vibrio* biofilm formation by a marine actinomycete strain A66. *Applied Microbiology and Biotechnology* 76(5):1137-1144.