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Research Article

SCREENING OF MICROFUNGI ISOLATED FROM ACIGÖL, TÜRKİYE FOR HYDROLYTIC ENZYMES, BIOACTIVE METABOLITES AND SILVER NANOPARTICLE PRODUCTION

Emine İRDEM[1*], Semra İLHAN[2], Ercan ÖZBİÇEN[3], Lira USAKBEK KYZY[3], Gamze TUNCA[3], Esma OCAK[1], Niyazi Can ZORLUER[4], Uğur ÇİĞDEM[1], Fatma AYVA SÖĞÜT[5], Rasime DEMİREL[5]

[1]Eskişehir Osmangazi University, Graduate School of Natural and Applied Sciences, Biotechnology and Biosafety Department, TR-26480 Eskişehir, TÜRKİYE
[2]Eskişehir Osmangazi University, Faculty of Science, Department of Biology, TR-26040 Eskişehir, TÜRKİYE
[3]Eskişehir Osmangazi University, Graduate School of Natural and Applied Sciences, Biology Department, TR-26480 Eskişehir, TÜRKİYE
[4]Eskişehir Osmangazi University, Faculty of Science, Programme of Biology, TR-26040 Eskişehir, TÜRKİYE
[5]Eskişehir Technical University, Faculty of Science, Department of Biology, TR-26000 Eskişehir, TÜRKİYE

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İskender Karatlı

****Corresponding Author:***
Emine İrdem
emineirdem@gmail.com

ORCID iDs of the authors:
Eİ. orcid.org/0000-0002-2955-298X
Sİ. orcid.org/0000-0002-3787-2449
EÖ. orcid.org/0000-0001-5646-6115
LUK. orcid.org/0000-0002-9424-4473
GET. orcid.org/0000-0002-4126-8340
EO. orcid.org/0000-0002-9085-4151
NCZ. orcid.org/0000-0002-2394-2194
UÇ. orcid.org/0000-0003-4790-494X
FAS. orcid.org/0000-0002-7072-2928
RD. orcid.org/0000-0001-6702-489X

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Abstract: Haloalkalitolerant fungi can survive in environments with high salt concentrations and pH values. The bioactive compounds produced under stressful conditions have potential biotechnological applications. In this study, 52 microfungi isolated from Acıgöl Lake in Türkiye, offering polyextreme conditions, were screened for some biotechnological properties. The antimicrobial and antioxidant activities of the isolates were determined using the agar diffusion and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods, respectively. Enzyme activities were determined by various methods using the agar diffusion technique. Synthesis of silver nanoparticles (AgNPs) was carried out using cell-free filtrate of microfungi. 40% of the isolates showed antimicrobial activity against at least one of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Candida albicans* ATCC 90028 used as the test microorganisms. *Penicillium dipodomyicola* showed the highest antibacterial activity against *E. coli* and *S. aureus*, while *P. brevicompactum* showed the highest activity against *C. albicans*. *Penicillium dipodomyicola* and *P. bilaiae* were found to have free radical scavenging activity of a level (90% and above) that can compete with positive control. All of the isolates with amylase activity belonged to *Aspergillus* and *Penicillium* and the most prominent three of them were *A. ochraceous, A. flavus* and *P. brevicompactum*. 55% of the isolates showed proteolytic activity, among which *A. alliaceus* had the highest activity. Almost all isolates (92%) showed lipolytic activity. *Aspergillus amstelodami, P. sizovae* and *P. solitum* had a significant level of lipolytic activity. 35% of the isolates showed cellulolytic activity with highest values *Cladosporium pseudocladosporioides, P. dipodomyicola* and *P. bilaiae*. Eight of the isolates carried out AgNP synthesis within 24 h. When all the results were evaluated, *Aspergillus amstelodami, A. ochraceus, Penicillium dipodomyicola*, and *P. brevicompactum* appeared to have the potential to serve in different industrial areas.

Özet: Haloalkalitolerant funguslar, tuz konsantrasyonunun ve pH değerinin yüksek olduğu ortamlarda yaşayabilirler. Stresli koşullar altında üretilen biyoaktif bileşiklerin potansiyel biyoteknolojik uygulamaları vardır. Bu çalışmada, poliekstrem koşullara sahip Acıgöl/Türkiye'den izole edilen 52 mikrofungus, bazı biyoteknolojik özellikler açısından taranmıştır. Antimikrobiyal ve antioksidan aktiviteler sırasıyla agar difüzyon ve 2,2-difenil-1-pikrilhidrazil (DPPH) radikal süpürücü etki yöntemi kullanılarak belirlenmiştir. Enzim aktiviteleri, agar difüzyon tekniğinin kullanıldığı çeşitli yöntemlerle belirlenmiştir. Gümüş nanopartikül (AgNP) sentezi, hücre içermeyen mikrofungus filtratı kullanılarak gerçekleştirilmiştir. İzolatların %40'ı test mikroorganizmalarından en az birine karşı antimikrobiyal aktivite göstermiştir. *Penicillium dipodomyicola, E. coli* ve *S. aureus*'a karşı

en yüksek antibakteriyel aktiviteyi gösterirken, *P. brevicompactum Candida albicans*'a karşı en yüksek aktiviteyi göstermiştir. *Penicillium dipodomyicola* ve *P. bilaiae*'nin pozitif kontrol ile rekabet edebilecek düzeyde (%90 ve üzeri) serbest radikal süpürücü aktiviteye sahip olduğu belirlenmiştir. Amilaz aktivitesine sahip izolatların tamamı *Aspergillus* ve *Penicillium* cinslerine ait olup, *A. ochraceous, A. flavus* ve *P. brevicompactum* en yüksek aktiviteye sahip türler olarak belirlenmiştir. İzolatların %55'i proteolitik aktivite gösterirken, *A. alliaceus* en yüksek aktiviteye sahip izolat olmuştur. Hemen hemen tüm izolatlar (%92) lipolitik aktivite göstermiştir. Özellikle *A. amstelodami, P. sizovae* ve *P. solitum* izolatlarının önemli düzeyde lipolitik aktiviteye sahip olduğu belirlenmiştir. İzolatların %35'i selülolitik aktivite göstermiş ve *Cladosporium pseudocladosporioides, P. dipodomyicola* ve *P. bilaiae* izolatlarında yüksek aktivite gözlenmiştir. İzolatların sekizi 24 saat içinde AgNP sentezini gerçekleştirmiştir. Tüm sonuçlar değerlendirildiğinde *Aspergillus amstelodami, A. ochraceus, Penicillium dipodomyicola* ve *P. brevicompactum* gibi izolatların farklı endüstriyel alanlarda hizmet verme potansiyeline sahip olduğu belirlenmiştir.

Introduction

Fungi are cosmopolitan organisms with single or multicellular eukaryotic cell structure and potentially play important roles in organic matter cycle and food chain dynamics. Although mainly terrestrial, they are components of almost all ecosystems. Fungi are found in various aquatic environments ranging from high mountain lakes to deep ocean but their presence in aquatic ecosystems has often been overlooked so far (Grossart *et al.* 2019). Therefore, what we already know about the diversity, biology and ecology of aquatic fungi is very little compared to terrestrial fungi. Recent studies have focused on uncovering fungal diversity in extreme environments such as poles, hot springs, salterns, dried-up rocks, dry deserts and areas with very low/high pH values. Some fungi can adapt to polyextreme environments accompanied by conditions such as hipersalinity, high/low temperature, variable pH and ultraviolet radiation. In most cases, fungi in such environments survive through their special adaptation mechanisms they have developed as a response to environmental driving forces. Most of the hipersaline environments are formed by evaporation of sea water and are called "Thalassohaline Waters". Sodium and chloride ions are dominant in the salt composition of sea water. Other hipersaline environments consist of dissolution of salts of continental origin. The ionic components of these environments, which are called "Athalassohaline Waters", are quite different from sea water. There are many athalassohaline lakes, for instance the Dead Sea in Israel, in the world (Oren 2002, Ventosa 2004). Acıgöl Lake, located in inner Aegean Region in Türkiye is the second largest athalassohaline lake in the world and is one of the most important sodium sulphate and NaCl production fields in the country. The lake creates a polyextreme environment for organisms with its environmental features such as pH shifting to alkaline with its ion diversity and density, exposure to sunlight and temperature change pattern in a wide range.

Hypersaline waters were thought to harbor only halophilic algae and bacteria and be completely devoid of fungi until the last few decades. Since then, halophilic and alkalophilic fungi that can survive in high saline and/or high alkaline environments have been isolated and characterized and were proved to be inhabitants of such environments (Gunde-Cimerman *et al.* 2000, Gunde-Cimerman *et al.* 2004, Hozzein *et al.* 2013, Wei & Zhang 2019, Orwa *et al.* 2020, Özgök & İlhan 2020). Further related studies showed that haloalkaliphilic fungi could be potential agents for bioremediation in salty and alkaline soils, and some halophilic fungi produced metabolites with antimicrobial, antioxidant and antiproliferative effects (Kis-Papo *et al.* 2001, Cantürk *et al.* 2017, Wei & Zhang 2018). Halophilic fungi has also been emphasized as important genetic pools for gene cloning (Zhang *et al.* 2018).

Haloalkaliphilic fungi are one of the extremophilic microorganisms that grow best under conditions of high salinity and extreme alkalinity. Due to their successful growth in hypersaline conditions and the combination of other harsh conditions, haloalkaliphilic/ haloalkalitolerant fungi can produce a variety of metabolites that can withstand harsh conditions. This shows that they are important target organisms in researches on determination of new strains with biotechnological potential to be used in industry and cases of environmental problems. Unlike traditional strains, haloalkaliphilic fungi and their metabolites have enormous usage potential in many biotechnology fields. In this study, we aimed to elucidate the potential of microfungi isolated from a salty and alkaline lake to be producers of industrially important products such as enzymes, bioactive metabolites, and nanoparticles.

Materials and Methods

Fungal Strains

52 fungal species within 9 genera [*Acremonium* (1), *Alternaria* (7), *Aspergillus* (13), *Cladosporium* (3), *Fusarium* (4), *Neocosmospora* (6), *Penicillium* (14), *Rhizopus* (1), *Trichoderma* (1), and unidentified (2) isolates] previously isolated in November 2019 from Acıgöl were included in the study. Morphological (phenotypic properties) and molecular (multi locus gene sequencing) methods were used in the identification of the species in the previous study (Ayva *et al.* 2021). Stock cultures of the isolates were maintained on Potato Dextrose Agar (PDA) and stored at +4°C.

Antimicrobial Activity

The agar diffusion method with modifications was used to determine the antimicrobial activities of the isolates (CLSI 2004, 2015). For this purpose, the bacterial strains *Escherichia coli* ATCC 25922 and *Staphylococcus*

aureus ATCC 29213, and the yeast *Candida albicans* ATCC 90028 were used as the test organisms.

Bacteria were incubated on Muller Hinton Broth (MHB) and the yeast on Sabouraud Dextrose Broth (SDB) at 37ºC for 24 hours. The concentration of cell suspensions was adjusted to the turbidity of the 0.5 McFarland standard (at 625 nm, 0.08 to 0.1 absorbance) with sterile saline water. Microorganism suspensions were spread onto 90 mm petri plates, including Mueller Hinton Agar (MHA) for the bacterial and Sabouraud Dextrose Agar (SDA) for the yeast, using sterile swabs.

The fungal isolates were incubated on PDA for 7 days at room temperature. Then, the agar plates were cut into small agar blocks (6 mm in diameter) using a sterile cork-borer and the blocks were placed on test plates inoculated with test microorganisms. After the plates were kept in the refrigerator for one hour, they were incubated at 37ºC for 24 hours. The clear zones around the agar blocks on the plates were considered inhibition of the test microorganisms. Diameters of inhibition zones were recorded in millimeter (mm). All assays were done in duplicate. Gentamicin (Bioanalyse, 10 mcg), Sulbactam+Ampicillin (Bioanalyse, 20 mcg) and Fluconazole (MP Biomedicals, 1.25mg/ml) were used as positive control.

Antioxidant Activity

The antioxidant activities of the fungal isolates were determined by modifying the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method developed by Sánchez-Moreno *et al.* (1998). Fungal isolates were incubated in an antioxidant liquid medium with content of 30.0 g/L sucrose, 1.0 g/L yeast extract, 1.0 g/L peptone, 0.5 g/L KCl, 0.01 g/L $FeSO_4.7H_2O$, 0.5 g/L $MgSO_4.7H_2O$ and 1.0 g/L K_2HPO_4 at 27°C, under static conditions for 7 days (Malpure *et al.* 2006).

3 ml DPPH (2×10^{-2} g/L) solution was added to 100 µl supernatant obtained after incubation and vortexed for 30 s. The reaction tubes, in triplicates, were wrapped in aluminum foil and kept at room temperature for 30 min in dark. Spectrophotometric measurements were done at 517 nm using CECIL 4002 spectrophotometer. Butylated hydroxytoluene (BHT) was used as positive control. Percentage inhibition calculated according to below given formula (Cantürk *et al.* 2017):

$$Inhibition\ \% = \frac{Control\ absorbance - Absorbance\ of\ sample}{Control\ absorbance} \times 100$$

Enzymatic Activity

All fungal isolates were screened for their ability to produce extracellular amylase, lipase, protease and celullase. The enzyme activity was determined in agar plate method and performed in duplicate. Regarding the size of the zone diameters, the degree of all activities was evaluated as low (+), medium (++) and high (+++).

Amylolytic enzyme assay: In order to determine the amylase production ability of the isolates, single point inoculation from activated cultures was made on petri dishes containing 20.0 g/L soluble starch, 1.0 g/L yeast extract, 1.0 g/L K_2HPO_4, 1.0 g/L $MgSO_4.7H_2O$, 20.0 g/L agar at 6.0 pH. After 7 days of incubation, Lugol's iodine solution (0.1% iodine; 1.0% potassium iodide w/v) was flooded on the plates and amylase production was observed as the appearance of zone of clearance against dark (bluish black) background (Fossi *et al.* 2009).

Lipolytic enzyme assay: A tributyrin-containing medium was used in determination of the lipolytic activity of the fungal isolates. The medium containing 5.0 g/L peptone, 3.0 g/L yeast extract, 20.0 g/L agar (pH: 6.0) was autoclaved at 121°C for 15 minutes, and then tributyrin was added 1/l (w/w) to medium and vortexed thoroughly. Then, the medium was poured into sterile petri dishes (Griebeler *et al.* 2011). Activated cultures were inoculated as single point on plates and incubated at 25°C for 7 days. At the end of the incubation, isolates forming clear zones around their colonies on the plates were considered to exhibit lipase activity.

Proteolytic enzyme assay: The method of Topal *et al.* (2000) was used with modifications for determination of the protease activities of the isolates. The medium containing 1.0 g/L K_2HPO_4, 0.5 g/L $MgSO_4\cdot7H_2O$, 0.5 g/L KCl, 16.0 g/L agar was autoclaved at 121°C and 15 min. After cooling to 60ºC, sterile milk was added to the medium so that the casein concentration was 3%, mixed and the medium was poured into sterile petri plates. The activated isolates were inoculated as single point to the center of petri plate and incubated at 25°C for 7 days. After incubation, the diameters of the clear zones formed around the colonies were measured.

Cellulolytic enzyme assay: Czapek-Dox agar (HKM, Catalog no: HCM122) (pH: 5.0) containing 1% carboxymethyl cellulose (Sigma Aldrich, Catalog no: C5678) was used to determine the cellulase activity. Activated isolates were inoculated into petri plates containing Czapek-Dox agar in a single point. Petri plates incubated at 27°C for 7 days were treated with 1% Congo red solution and then 1M NaCl solution. Clear zones formed around the colonies showed the presence of cellulolytic activity (Kluepfel 1988).

Biosynthesis of silver nanoparticles (AgNPs)

Silver nanoparticles were synthesized according to the modified version of the method described in Ottoni *et al.* (2017). 100 ml Malt Glucose Yeast Peptone (MGYP) medium (3.0 g/L malt extract, 10.0 g/L glucose, 3.0 g/L yeast extract, and 5.0 g/L peptone) prepared in 500 ml flasks was inoculated with spores collected from the active culture surfaces with 0.1% Tween 80. The flasks were incubated at 27°C and 140 rpm for 96h. Mycelia were filtrated through filter paper and washed three times with sterile distilled water. 10 g (wet weight) of biomass were suspended in 100 ml sterile distilled water and incubated at 27°C with agitation rate of 140 rpm for 48 h. After the incubation, the filtrate obtained by passing the water suspended with

biomass through filter paper were treated with 0.5 mM silver nitrate (Sigma Aldrich) solution in a flask and incubated at room temperature with agitation in dark for 24 h. The filtrate without silver nitrate solution was also run as control. After 24 h of reaction, a color change to yellowish brown occurring in the solution indicated the formation of AgNPs. The presence of AgNPs was confirmed by UV-VIS spectroscopy. The solutions were scanned in 200-800 nm range and the peaks between 420-430 nm indicated the presence of AgNPs (Ottoni *et al.* 2017).

Results

Antimicrobial activity

The results obtained with the isolates showing significant antimicrobial activity are given in Table 1. Forty percent of the isolates showed activity against at least one of the test microorganisms. Among these isolates, 52% showed antibacterial, 33% showed antifungal activities, and 14% inhibited the growth of all test organisms. Three strains of *P. dipodomyicola* (AG38, AG41 and AG45) showed the highest antibacterial activity against test bacteria, while *P. brevicompactum* AG36 showed the highest activity against *C. albicans* (Table 1 and Fig. 1).

Table 1. The isolates showing significant antimicrobial activity and their inhibition zone diameter.

Isolates	Zone Diameter (mm)		
	E. coli ATCC 25922	*S. aureus* ATCC 29213	*C. albicans* ATCC 90028
Alternaria alternata AG10	-	11	-
A. alternata AG13	-	15	-
A. alternata AG 26	-	22	-
A. alternata AG14	-	15	-
A. alternata AG15	-	11	-
Aspergillus terreus AG29	-	23	11
A. terreus AG30	-	17	10
A ochraceus AG31	-	11	-
A. ochraceus AG52	16	13	17
Neocosmospora keratoplastica AC2	15	-	-
N. solani AG6	12	11	-
Fusarium equiseti AG9	-	-	11
Penicillium bilaiae AG 44	-	-	10
P. brevicompactum AG36			25
P. brevicompactum AG47	-	-	19
P. brevicompactum AG39	-	-	20
P. brevicompactum AG43	-	-	18
P. brevicompactum AG42	-	-	18
P. dipodomyicola AG38	17	21	-
P. dipodomyicola AG41	20	30	-
P. dipodomyicola AG45	25	29	-
Gentamicin 10 mcg	21		
Sulbactam+Ampicillin 20 mcg		-	
Fluconazole 1.25 mg/ml			22

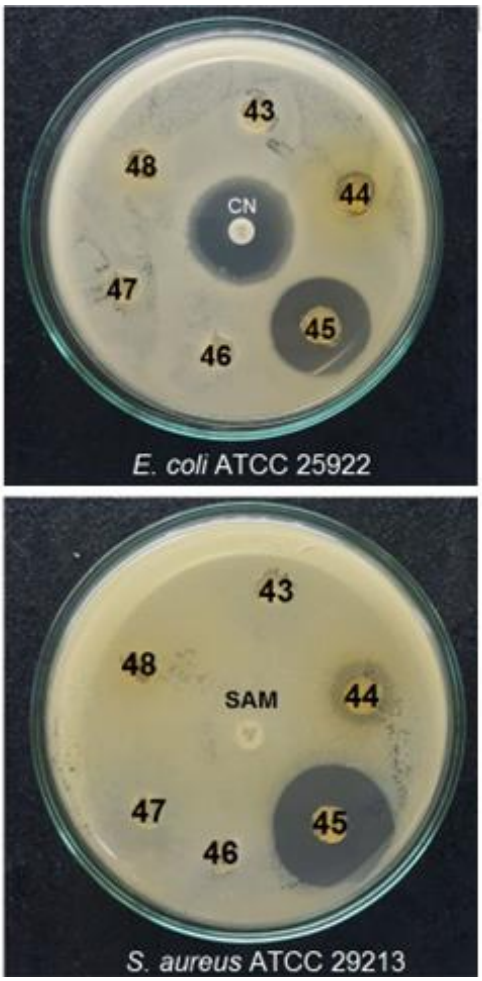

Fig. 1. Zones of inhibition showing the effect of *P. dipodomyicola* AG45 (number 45) on test bacteria, CN; Gentamicin, SAM; Sulbactam+Ampicillin

Antioxidant activity

Free radical scavenging activities of the isolates were calculated in percent. Only four isolates were found to have a higher inhibition rate than the positive control (Table 2).

Enzyme activity

Findings regarding amylase, lipase, protease and cellulase activities of the fungal isolates are given in Table 3. While 5 of 52 isolates showed activity in terms of all enzymes screened, no activity was detected in 3 isolates. The results revealed that the isolates had 92% lipolytic activity, 55% proteolytic activity, 35% cellulolytic activity and 25% amylolytic activity.

Table 2. The isolates showing significant antioxidant activity and their inhibition rates.

Fungal isolates	Inhibition %
P. dipodomyicola AG38	97
P. dipodomyicola AG41	96
P. dipodomyicola AG45	96
P. bilaiae AG44	96
BHT	80

Table 3.Enzyme activity levels of the fungal isolates.

Fungal strain	Amylolytic Activity	Lipolytic Activity	Proteolytic Activity	Cellulolytic Activity
Acremonium sclerotigenum AG58	ND	+	+++	ND
Alternaria alternata AG26	ND	+	ND	ND
A. alternata AG10	ND	+	+	ND
A. alternata AG11	ND	+	+	+
A. alternata AG12	ND	++	ND	+
A. alternata AG13	ND	+	ND	ND
A. alternata AG14	ND	++	+	ND
A. alternata AG15	ND	ND	ND	ND
Aspergillus flavus AG35	+++	+	+++	ND
A. alliaceus AG33	+	+	+++	ND
A. pseudoglaucus AG50	ND	++	ND	ND
A. intermedius AG51	ND	++	ND	+
A. intermedius AG53	ND	++	ND	+
A. amstelodami AG54	ND	+++	ND	+
A. amstelodami AG55	ND	++	ND	ND
A. cristatus AG48	ND	++	ND	ND
A. ochraceus AG31	+++	++	+	ND
A. ochraceus AG52	ND	++	+	ND
A. terreus AG29	ND	+	++	ND
A. terreus AG30	+	+	+++	ND
A. tubingensis AG32	ND	++	++	ND
Cladosporium acalyphae AG23	ND	++	+	+
C. cladosporioides AG25	ND	++	+	++
C. pseudocladosporioides AG24	ND	++	+	+++
Fusarium sp. AG7	ND	+	ND	ND
F. equiseti AG8	ND	+	ND	ND
F. fujikuroi AG4	ND	+	ND	ND
F. equiseti AG9	ND	+	ND	ND
Neocosmospora keratoplastica AG2	ND	+	++	ND
N. keratoplastica AG22	ND	ND	+	ND
N. solani AG1	ND	+	ND	ND
N. solani AG3	ND	ND	ND	ND
N. solani AG5	ND	+	+	ND
N. solani AG6	ND	+	+	ND
Isolate not identified AG27	ND	ND	ND	ND
Isolate not identified AG49	ND	++	ND	ND
Penicillium bilaiae AG44	++	+	+	+++
P. brevicompactum AG36	ND	++	+	+
P. brevicompactum AG39	++	++	+	+
P. brevicompactum AG42	++	++	+	+
P. brevicompactum AG43	+++	++	+	+
P. brevicompactum AG47	++	++	++	+
P. chrysogenum AG57	ND	+	ND	ND
P. solitum AG40	++	+++	++	ND
P. dipodomyicola AG45	+	++	ND	++
P. dipodomyicola AG38	++	++	ND	+++
P. dipodomyicola AG41	ND	++	+	+++
P. sizovae AG37	ND	+++	+	+
P. sizovae AG46	++	+++	+	ND
P. sizovae AG56	+	+++	+	ND
Rhizopus arrhizus AG16	ND	+	ND	ND
Trichoderma harzianum AG28	ND	+	ND	ND

Zone diameter (mm): **Amylolytic Activity** 1-2 (+) Low; 3-5 (++) Medium; 6-8 (+++) High; **Lipolytic Activity** 1-10 (+) Low; 11-23 (++) Medium; 24-37 (+++) High; **Proteolytic Activity** 1-5 (+) Low; 6-11 (++) Medium; 12-17 (+++) High; **Cellulolytic Activity** 1-10 (+) Low; 11-15 (++) Medium; 16-20 (+++) High; ND: Not Detected

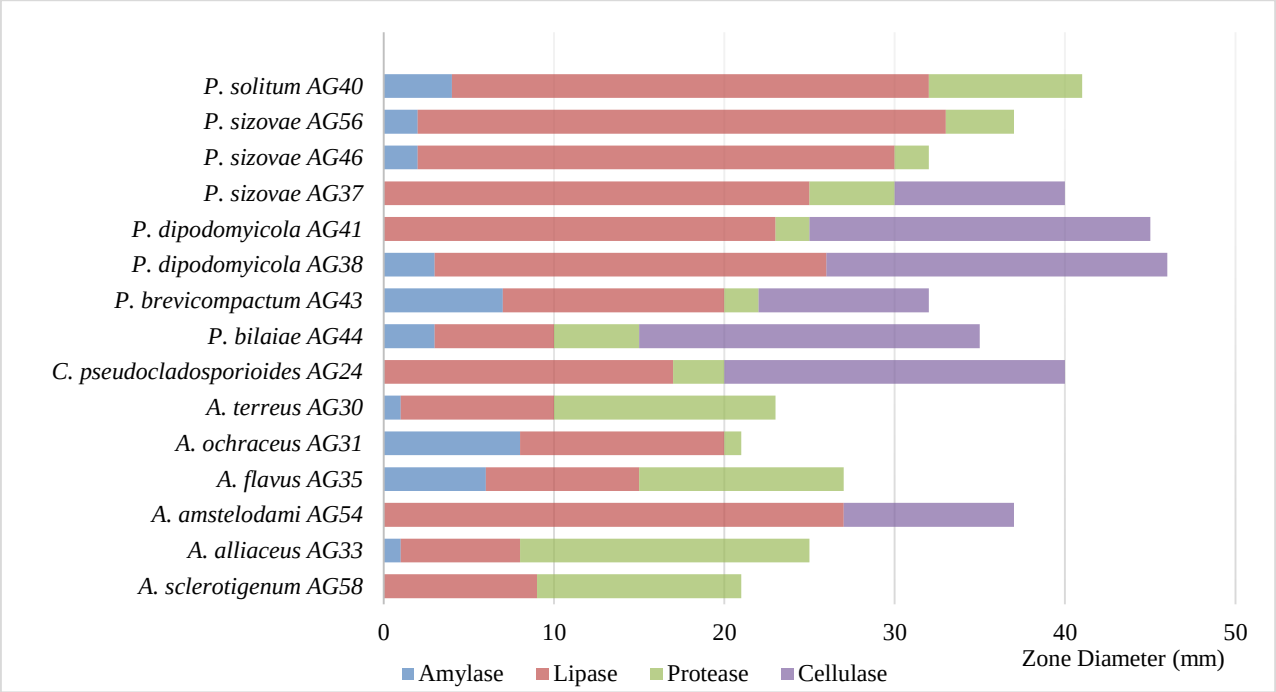

Fig. 2. Prominent isolates with high activity for at least one enzyme.

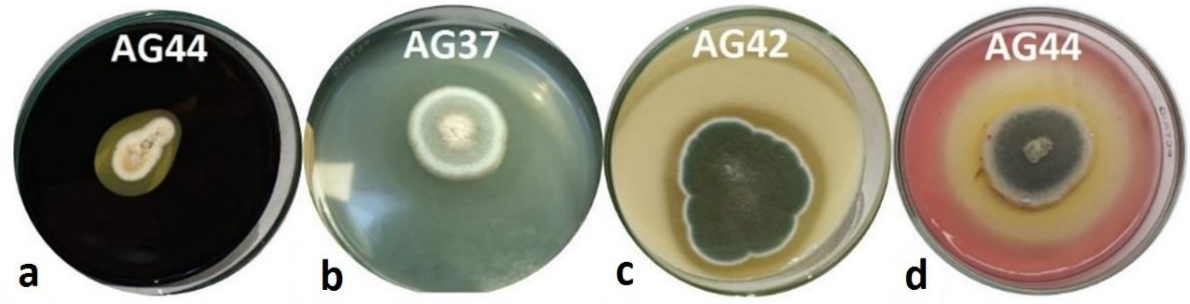

Fig. 3. Representative Petri dishes showing enzyme activities of some isolates. **a.** Amylolytic activity, **b.** lipolytic activity, **c.** proteolytic activity, **d.** cellulolytic activity.

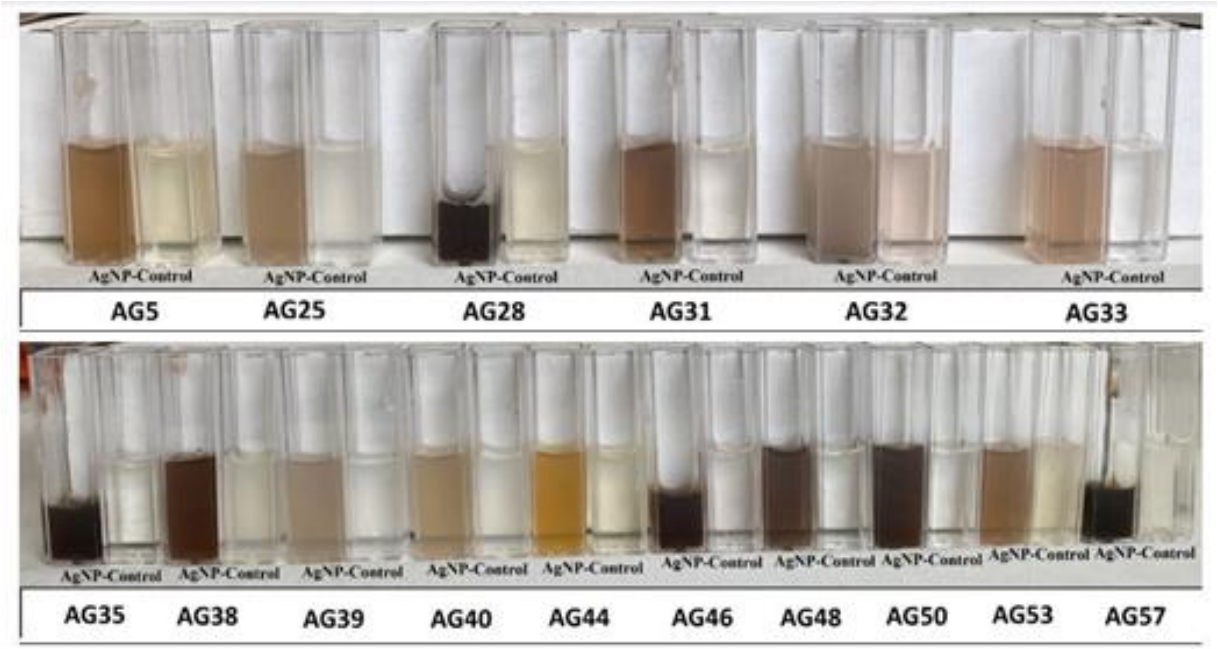

Fig. 4. The color change observed in the filtrates after adding $AgNO_3$.

Prominent isolates with high activity (+++) for at least one enzyme are compared in Fig. 2. The screening performed with four enzymes revealed that the isolates showing high activity in general are members of the *Penicillium*, *Aspergillus*, *Acremonium* and *Cladosporium*.

The evaluation of the results showed that the highest number of isolates with amylase activity was in the genus *Penicillium*, followed by *Aspergillus* (Table 3). The isolates with the highest amylase activity were *A. ochraceous* AG31, *P. brevicompactum* AG43 and *A. flavus* AG35, respectively. Amylase activity was not detected in isolates within the other genera screened (Fig. 3).

Lipolytic activity was detected in 92% of the isolates (Table 3). *Aspergillus amstelodami* AG54, *P. sizovae* AG37, AG46, AG56 and *P. solitum* AG40 isolates showed high lipase activity (Fig. 3). The highest activity was measured in *P. sizovae* AG56 isolate with a zone diameter of 15.5 mm.

Fifty six percent of the isolates showed proteolytic activity (Table 3). The highest activity was measured in *A. alliaceus* AG33 isolate with a zone diameter of 33 mm, followed by the isolates *A. terreus* AG30, *A. flavus* AG35 and *A. sclerotigenum* AG58.

Thirty five percentof the isolates showed cellulolytic activity (Table 3). Isolates with high cellulase activity were *C. pseudocladosporioides* AG24, *P. dipodomyicola* AG38, AC41 and *P. bilaiae* AG44 (Fig. 3).

Biosynthesis of silver nanoparticles

The UV-VIS measurements of the filtrates, which were left to incubate for 24 hours by adding 0.5 mM $AgNO_3$, were evaluated with a peak in the wavelength range of 200-800 nm. Eight isolates, *A. flavus* AG35, *A. pseudoglaucus* AG50, *A. intermedius* AG53, *P. bilaiae* AG44, *P. chrysogenum* AG57, *P. dipodomycola* AG38, *P. sizovae* AG46 and *T. harzianum* AG28, carried out AgNP synthesis within 24 h. In addition, color change was observed in all isolates left to incubation for longer, and they formed a peak in the 420-480 nm band (Figs 4-5).

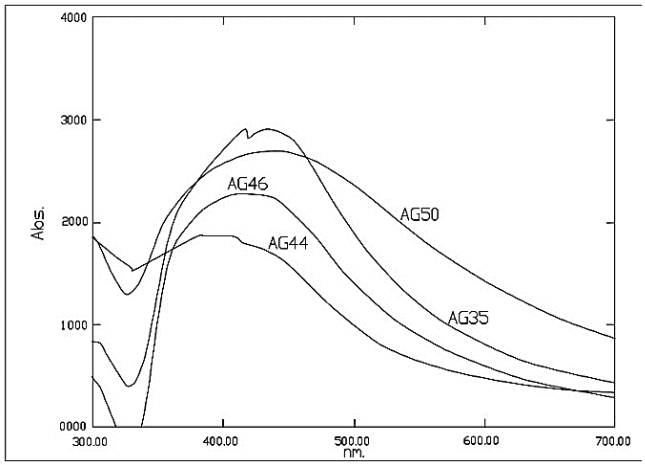

Fig. 5. The absorbances in UV-VIS of some filtrates left to incubation for 24 hours by adding 0.5 mM $AgNO_3$.

Discussion

The biotechnological potentials of 52 isolates from the *Trichocomaceae* family were evaluated by testing their antimicrobial activity, antioxidant activity, ability to produce the extracellular enzymes amylase, lipase, protease and cellulase), and AgNP synthesizing abilities. In general, haloalkalophilic fungi are an important source of polyextremophilic metabolites. These metabolites, extremozymes, are stable in extreme conditions and have pronounced activity and therefore have remarkable biotechnological applications. Although fungi have a similar metabolic diversity with other microorganisms, more attention should be drawn to their ease of cultivation.

We initially screened all 52 isolates for their antimicrobial activities, and it determined that 39% of the isolates showed an inhibitory effect against at least one test microorganism used. Among them, *A. alternata* isolates (AG10, 13, 26, 14, 15) showed an inhibitory effect against the Gram-positive bacteria *S. aureus* with zone diameters varying between 11-20 mm. Studies on the antimicrobial activity of *A. alternata* metabolites reported significant antibacterial effects against Gram-positive bacteria. In a recent study, seven of the tested compounds from the endophyte *A. alternata* 13A isolated from a saline lake in Sahara Desert were reported to be more effective against Gram-positive strains than Gram-negative strains (Qader *et al.* 2021). Also, Chandra *et al.* (2021) reported that the extract from *A. alternata* was capable of inhibition two Gram positive bacteria, *B. subtilis* and *S. aureus*.

Aspergillus and *Penicillium* which found in soil and saline environments are source of bioactive compounds. It has been reported that *Aspergillus* species have compounds with strong antibacterial effects (Al-Fakih & Almaqtri 2019). *Aspergillus* species are capable of production a large number of natural bioactive metabolites (Lee *et al.* 2013). In the present study, *A. ochraceus* and *A. terreus* isolates showed both antibacterial and antifungal activities. There are studies that elucidate the structure of metabolites with strong antimicrobial activity obtained from these *Aspergillus* species. The endophytic fungus *A. ochraceus* displayed remarkable antimicrobial activity in the study of Attia *et al.* (2020). Asperteramide from the marine-derived fungus *A. terreus* was reported to be responsible from inhibition of *Bacillus cereus* (Bunbamrung *et al.* 2020). Of the active *Penicillium* species, *P. dipodomyicola* isolates showed only antibacterial activity, while *P. brevicompactum* isolates showed antifungal activity. The highest activities against *E. coli* and *S. aureus* were produced by *P. dipodomyicola* AG45 and AG41, respectively, and the highest activity against *C. albicans* was produced by *P. brevicompactum* AG36. The genus *Penicillium* is commonly found in saline environments and has received great attention due to the presence of secondary metabolites. Most of the bioactive secondary metabolites reported so far was related with *Penicillium* (Vansteelandt *et al.* 2012, Koul & Singh 2017, Farha & Hatha 2019). In this respect, *Aspergillus* and *Penicillium*

species may be a potential source of metabolites that may help treating of infectious diseases with increasing resistance to currently used antibiotics and may provide alternative medical therapy.

The modified DPPH method was used to determine the antioxidant activity of the isolates (Cantürk *et al.* 2017). Measuring the free radical scavenging effect as percent inhibition is a common situation in the literature. The higher the percentage value, the higher the activity. Four isolates of the genus *Penicillium* showed free radical scavenger effect above BHT value 80% used as a standard. In an antioxidant activity study, the inhibition rate of *P. chrysogenum* was 63%, while the DPPH inhibition of *P. dipodomyicola* was 97% (Sikandar *et al.* 2020). Production optimization studies can increase the DPPH radical scavenging ability (Chandra & Arora 2017). Our finding indicates that *P. dipodomyicola* isolates may be evaluated as natural antioxidant agents.

Polyextremophilic microfungi are potential sources of enzymes for industrial applications under unfavorable conditions such as high salt concentration, alkaline pH, and presence of organic solvents. Fungal enzymes can also be secreted extracellularly. Microfungi isolated from hypersaline and alkaline waters were screened for amylase, lipase, protease and cellulase activities using solid media. This method used is an indication that the detected enzymes are secreted out of the cell. The extracellular nature of the enzyme produced in industrial production provides an important advantage. In this study, it was determined that 49 of the 52 isolates tested had at least one enzyme activity. Of these, 4 strains of *P. brevicompactum* and 1 strain of *P. bilaiae* showed activity for all enzymes tested.

Amylase is an important enzyme that catalyzing starch into its monomers and is widely used industrially (Bodade & Lonkar 2022). Particularly, effective amylase producing species were reported to be members of *Aspergillus* and *Penicillium* (Kathiresan & Manivannan 2006, Demirel *et al.* 2008, Gouda & Elbahloul 2008, Gopinath *et al.* 2017, Souza *et al.* 2020). In our study, isolates which were determined to show amylase activity were also members of *Aspergillus* and *Penicillium*. *Aspergillus flavus*, *A. ochraceus*, and *P. brevicompactum* were the best amylase producers. Non-toxic fungal amylases are used in many industrial productions, including the food and pharmaceutical industries (Niknejad *et al.* 2013). The data obtained from screening studies with fungi isolated from polyextreme environments will form the basic tool for next steps of production optimization, purification, and detailed characterization. Reports on the purification and characterization of amylases from halophilic fungi are limited (Ali *et al.* 2015).

Lipases catalyze the hydrolysis of triacylglycerols and the synthesis of esters from glycerol and long-chain fatty acids. Microbial lipases are of particular interest industrially because of their stability to extreme temperatures and pH, as well as their broad substrate specificity. Their versatility makes them important for a variety of biotechnological applications, mainly in biodiesel processing, pharmaceutical, food and detergent industries (Pérez *et al.* 2019). The lipolytic enzyme activities of the strains were determined according to the zone formation in solid medium containing tributyrin as the substrate. This method is frequently used in screening studies (Griebeler *et al.* 2011, Patel & Shah 2020). Most of our isolates (92%) showed lipolytic activity, and the genera containing the species with the highest activity were *Aspergillus* and *Penicillium*. The highest activity was observed in *A. amstelodami*, *P. solitum* and *P. sizovae*. Wadia and Jain (2017) found in their study that only five fungal species, *A. niger*, *A. flavus*, *Penicillium* sp., *Alternaria* sp. and *Trichoderma* sp. have the potential to produce lipase on Tri Butyrin agar (TBA) agar plates. The authors reported that the diameter of the zone formed due to the lipolytic activity increased as the incubation time increased. In another study, *A. fumigatus* was reported as the species with maximum lipase activity (Mehta *et al.* 2018).

Proteases are a large group of enzymes that degrade proteins or peptides which are frequently used in industries such as detergent, leather, food, and medicine (Salwan & Sharma 2020). Although the number of studies on fungal proteases is increasing day by day, the full potential of extreme fungal proteases for industrial production waits to be exploited (Pavlukova *et al.* 1998, Sabotič *et al.* 2007, Yike 2011, Chandrasekaran *et al.* 2015, Sun *et al.* 2021). Many filamentous fungal species are known to produce proteases. Among these, members of the genus *Aspergillus* are at the forefront, and were also in the first place in terms of proteolytic activity in the present study. *Aspergillus alliaceus*, *A. terreus*, *A. flavus* and *A. sclerotium* are species with high protease activity. As the evaluation of many researchers on industrial protease production, it is seen that *Aspergillus* species come to the fore (Topal *et al.* 2000).

Fungal cellulases are well-studied enzymes and are frequently used in various industrial processes. Cellulases attract more attention due to their contribution to the biofuel production process. In the present study, 35% of the isolates showed cellulolytic activity and *C. pseudocladosporioides*, *P. dipodomyicola* and *P. bilaiae* isolates with high activity had the potential to be sources of cellulase enzymes. In recent years, cellulase production and optimization have been performed from different microfungal species such as *A. terreus*, *Chaetomium* sp. *A. niger*, *P. funiculosum*, *A. tubingensis* and *T. longibrachiatum* (de Carvalho *et al.* 2014, Sohail *et al.* 2016, El-Nahrawy *et al.* 2017, Pachauri *et al.* 2017). In another recent study, 3 different cellulase activities were reported from 3 fungal isolates, *P. funiculosum*, *C. cladosporioides*, *F. verticillioides*, isolated from Moringa biomass (Vázquez-Montoya *et al.* 2020).

Differences in the degree of activities were observed among strains of the same species, a case which may be

attributed to small genetic differences between the strains and to experimental factors. The thickness of the agar plates used, and the incubation time are particularly important in agar diffusion method and have the potential to affect the results, albeit slightly.

Screening studies used to determine to current potential of the isolates are very important in transforming the isolates into a strain that can be used in industrial production via production optimization and experimental designs (Raghav *et al.* 2022). We conclude that the hydrolytic activities of the strains used in the present study will increase after optimization of the test conditions in further studies.

The combination of nanotechnology and sustainable chemistry is very popular in the production of biocompatible metallic nanoparticles. The applications of AgNPs synthesized using many microorganisms, including fungi, in medical and agricultural fields are being investigated (Ahmed *et al.* 2018). AgNPs are the subject of research as antiviral, antimicrobial, antioxidant, antitumor, antibiofilm anti-inflammatory agents. When AgNP production abilities of the isolates were screened, *A. flavus AG35*, *A. pseudoglaucus* AG50, *P. dipodomyicola* AG38 and *P. sizovae* AG46 came to the fore. Although the methods used in the microbiological production of AgNP are similar, Ag ions are the primary requirement for the synthesis of AgNPs, which can be obtained from water-soluble silver salts, especially in the production using metal ion solution. However, aqueous $AgNO_3$ solution, with Ag ion concentration range of 0.1 - 10 mM (most commonly 1 mM), has been used by most researchers (Srikar *et al.* 2016). However, the factors that greatly affect the results here are the amounts of filtrate and $AgNO_3$ used and the ambient conditions where they come together. In this study, the final concentration was 0.5mM $AgNO_3$ using 100 ml of filtrate. The absorbance values of the filtrates expressing AgNP synthesis were quite high (Fig. 5). UV measurements were made after 24 hours of incubation. In another similar study, Verma *et al.* (2010) obtained very successful results by incubating 10 ml of filtrate with 90 ml of 1 mM $AgNO_3$ solution during 48 hours incubation period.

In this study, a scan was performed to understand the presence of AgNP based on color change and UV-VIS absorbance measurements. The isolates that stand out in the obtained results are seen as potential AgNP producer candidates. It is predicted that this type of polyextremophilic/tolerant microorganisms may provide inexpensive strategies in the industrial production of nanoparticles in terms of both physical conditions and medium components (Romano *et al.* 2022). In future studies, nanoparticle properties and yield can be determined by optimizing the production conditions of each fungal isolate as AgNP producer.

In conclusion, we report here the biotechnological application potential of microfungi isolated from Acıgöl for the first time. Haloalkalophilic fungi are rich sources for new natural products with various biological activities. It was determined that all of the isolates had at least one of the traits screened. Strains with high enzymatic and/or antimicrobial activity may be excellent candidates for use in suitable industries. The polyextremophilic nature of the strains makes them unique. The use of natural metabolites that can be obtained from them in food, medicine, detergent, etc. industries will be environmentally friendly alternatives that will rival the harmful chemicals commonly used in many products. Finally, this study shows that polyextremophilic fungi can become valuable tools in industrial and environmental biotechnology.

Studies on the screening, characterization and production optimization of fungal-derived enzymes are rapidly continuing to discover enzymes suitable for industrial needs. Because of their enormous genetic and biochemical diversity, extremophilic fungal strains isolated from Acıgöl can be seen as promising new enzyme sources with potential technological applications.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study.

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References

1. Ahmed, A.-A., Hamzah, H. & Maaroof, M. 2018. Analyzing formation of silver nanoparticles from the filamentous fungus *Fusarium oxysporum* and their antimicrobial activity. *Turkish Journal of Biology*, 42(1): 54-62.
2. Al-Fakih, A.A. & Almaqtri, W.Q.A. 2019. Overview on antibacterial metabolites from terrestrial *Aspergillus* spp. *Mycology*, 10(4): 191-209.
3. Ali, I., Akbar, A., Anwar, M., Prasongsuk, S., Lotrakul, P. & Punnapayak, H. 2015. Purification and characterization of a polyextremophilic α-Amylase from an obligate halophilic *Aspergillus penicillioides* isolate and its potential for souse with detergents. *Biomed Research International*, 1-8. https://doi.org/10.1155/2015/245649
4. Attia, E.Z., Farouk, H.M., Abdelmohsen, U.R. & Mo'men, H. 2020. Antimicrobial and extracellular oxidative enzyme activities of endophytic fungi isolated from alfalfa

(*Medicago sativa*) assisted by metabolic profiling. *South African Journal of Botany*, 134: 156-162.

5. Ayva, F., Demirel, R., İlhan, S., Usakbek Kyzy, L., Çiğdem, U., Zorluer, N., İrdem, E., Özbiçen, E., Ocak, E. & Tunca, G. 2021. Haloalkalitolerant and Haloalkaliphilic Fungal Diversity of Acıgöl/Turkey. *Mantar Dergisi*, 12(1): 33-41.

6. Bodade, R. & Lonkar, K. 2022. Extremophilic Fungal Amylases: Screening, Purification, Assay, and Applications, pp. 465-487. In: Sahay, S. (ed.). *Extremophilic Fungi*, Springer, Singapore, 748 pp.

7. Bunbamrung, N., Intaraudom, C., Dramae, A., Komwijit, S., Laorob, T., Khamsaeng, S. & Pittayakhajonwut, P. 2020. Antimicrobial, antimalarial and anticholinesterase substances from the marine-derived fungus *Aspergillus terreus* BCC51799. *Tetrahedron*, 76(41): 131496. https://doi.org/10.1016/j.tet.2020.131496

8. Cantürk, Z., Kocabıyık, E., Öztürk, N. & İlhan, S. 2017. Evaluation of antioxidant and antiproliferative metabolites of *Penicillium flavigenum* isolated from hypersaline environment: Tuz (Salt) Lake by Xcelligence technology. *Microbiology*, 86(3): 346-354. https://doi.org/10.1134/S0026261717030055

9. Chandra, H., Kumari, P., Prasad, R., Gupta, S.C. & Yadav, S. 2021. Antioxidant and antimicrobial activity displayed by a fungal endophyte *Alternaria alternata* isolated from Picrorhiza kurroa from Garhwal Himalayas, India. *Biocatalysis and Agricultural Biotechnology*, 33: 101955.

10. Chandra, P. & Arora, D.S. 2017. Antioxidant potential of two strains of *Aspergillus wentii* isolated from soil of different areas of Punjab, India and its optimization using different statistical approaches. *Journal of Microbiology and Biotechnology Research*, 4(4): 31-41.

11. Chandrasekaran, S., Kumaresan, S.S.P. & Manavalan, M. 2015. Production and optimization of protease by filamentous fungus isolated from paddy soil in Thiruvarur District Tamilnadu. *Journal of Applied Biology and Biotechnology*, 3(6): 066-069.

12. CLSI. 2004. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts *CLSI document M44-A*. Approved Guideline: Clinical and Laboratory Standards Institute.

13. CLSI. 2015. Performance Standards for Antimicrobial Disk Susceptibility Tests *CLSI document M02-A12*. Approved Standard-Twelfh Edition: Wayne, PA: Clinical and Laboratory Standards Institute.

14. de Carvalho, M.L.d.A., Carvalho, D.F., de Barros Gomes, E., Maeda, R.N., Santa Anna, L.M.M., de Castro, A.M. & Pereira Jr, N. 2014. Optimisation of cellulase production by *Penicillium funiculosum* in a stirred tank bioreactor using multivariate response surface analysis. *Enzyme Research*, 2014. https://doi.org/10.1155/2014/703291

15. Demirel, R., Yılmaz Sarıözlü, N. & İlhan, S. 2008. Production of fungal amylase enzyme from *Aspergillus* species in solid-state culture, *Journal of Biotechnology*, 136: S31.

16. El-Nahrawy, S., Metwally, M., El-Kodoos, A., Rizk, Y., Belal, E.-S.B., Shabana, S.A. & El-Refai, I.M. 2017. Optimization of culture conditions for production of cellulase by *Aspergillus tubingensis* KY615746 using rice straw waste. *Environment, Biodiversity and Soil Security*, 1(2017): 177-189.

17. Farha, A.K. & Hatha, A.M. 2019. Bioprospecting potential and secondary metabolite profile of a novel sediment-derived fungus *Penicillium sp*. ArCSPf from continental slope of Eastern Arabian Sea. *Mycology*, 10(2): 109-117. https://doi.org/10.1080/21501203.2019.1572034

18. Fossi, B.T., Tavea, F., Jiwoua, C. & Ndjouenkeu, R. 2009. Screening and phenotypic characterization of thermostable amylases producing yeasts and bacteria strains from some Cameroonian soils. *African Journal of Microbiology Research*, 3(9): 504-514.

19. Gopinath, S.C., Anbu, P., Arshad, M.M., Lakshmipriya, T., Voon, C.H., Hashim, U. & Chinni, S.V. 2017. Biotechnological processes in microbial amylase production. *Biomed Research International*, 2017, 1272193. https://doi.org/10.1155/2017/1272193

20. Gouda, M. & Elbahloul, Y. 2008. Statistical optimization and partial characterization of amylases produced by halotolerant *Penicillium sp*. *World Journal of Agricultural Sciences*, 4(3): 359-368.

21. Griebeler, N., Polloni, A.E., Remonatto, D., Arbter, F., Vardanega, R., Cechet, J.L., Di Luccio, M., de Oliveira, D., Treichel, H. & Cansian, R.L. 2011. Isolation and screening of lipase-producing fungi with hydrolytic activity. *Food and Bioprocess Technology*, 4(4): 578-586.

22. Grossart, H.-P., Van den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M. & Rojas-Jimenez, K. 2019. Fungi in aquatic ecosystems. *Nature Reviews Microbiology*, 17(6): 339-354.

23. Gunde-Cimerman, N., Zalar, P., de Hoog, S. & Plemenitaš, A. 2000. Hypersaline waters in salterns–natural ecological niches for halophilic black yeasts. *FEMS Microbiology Ecology*, 32(3): 235-240. https://doi.org/10.1111/j.1574-6941.2000.tb00716.x

24. Gunde-Cimerman, N., Zalar, P., Petrovič, U., Turk, M., Kogej, T., de Hoog, G.S. & Plemenitaš, A. 2004. Fungi in salterns, pp. 103-113. In A. Ventosa (ed.), *Halophilic Microorganisms*, Springer Berlin Heidelberg, Berlin, Heidelberg, 350 pp.

25. Hozzein, W.N., Ali, M.I.A. & Ahmed, M.S. 2013. Antimicrobial activities of some alkaliphilic and alkaline-resistant microorganisms isolated from Wadi Araba, the eastern desert of Egypt. *Life Science Journal*, 10(4): 1823-1828.

26. Kathiresan, K. & Manivannan, S. 2006. Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. *African Journal of Biotechnology*, 5(10): 829-832.

27. Kis-Papo, T., Grishkan, I., Oren, A., Wasser, S. & Nevo, E. 2001. Spatiotemporal diversity of filamentous fungi in the hypersaline Dead Sea. *Mycological Research*, 105(06): 749-756.

28. Kluepfel, D. 1988. Screening of prokaryotes for cellulose- and hemicellulose-degrading enzymes, *Methods in Enzymology*, 160: 180-186.

29. Koul, M. & Singh, S. 2017. *Penicillium spp*.: prolific producer for harnessing cytotoxic secondary metabolites.

Anticancer Drugs, 28(1): 11-30. https://doi.org/10.1097/CAD.0000000000000423

30. Lee, Y.M., Kim, M.J., Li, H., Zhang, P., Bao, B., Lee, K.J. & Jung, J.H. 2013. Marine-derived *Aspergillus* species as a source of bioactive secondary metabolites. *Marine Biotechnology*, 15(5): 499-519.

31. Malpure, P.P., Shah, A.S. & Juvekar, A.R. 2006. Antioxidant and anti-inflammatory activity of extract obtained from *Aspergillus candidus* MTCC 2202 broth filtrate. *Indian Journal of Experimental Biology*, 44(6): 468.

32. Mehta, A., Bodh, U. & Gupta, R. 2018. Isolation of a novel lipase producing fungal isolate *Aspergillus fumigatus* and production optimization of enzyme. *Biocatalysis and Biotransformation*, 36(6): 450-457.

33. Niknejad, F., Moshfegh, M., Najafzadeh, M.J., Houbraken, J., Rezaei, S., Zarrini, G., Faramarzi, M.A. & Nafissi-Varcheh, N. 2013. Halotolerant ability and α-amylase activity of some saltwater fungal isolates. *Iranian Journal of Pharmaceutical Research: IJPR*, 12: 113.

34. Oren, A. 2002. *Halophilic microorganisms and their environments*: Kluwer Academic Publishers, XXI, 575 pp.

35. Orwa, P., Mugambi, G., Wekesa, V. & Mwirichia, R. 2020. Isolation of haloalkaliphilic fungi from Lake Magadi in Kenya. *Heliyon*, 6(1): e02823. https://doi.org/10.1016/j.heliyon.2019.e02823

36. Ottoni, C.A., Simoes, M.F., Fernandes, S., Dos Santos, J.G., da Silva, E.S., de Souza, R.F.B. & Maiorano, A.E. 2017. Screening of filamentous fungi for antimicrobial silver nanoparticles synthesis. *Amb Express*, 7(1): 31. https://doi.org/10.1186/s13568-017-0332-2

37. Özgök, Ö. & İlhan, S. 2020. Diversity and distribution of *Dematiaceous* fungi in Çamaltı Saltern in İzmir province, Turkey. *Mantar Dergisi*, 11(1): 29-39. doi: 10.30708/mantar.645828

38. Pachauri, P., Aranganathan, V., More, S., Sullia, S. & Deshmukh, S. 2017. Purification and characterization of cellulase from a novel isolate of *Trichoderma longibrachiatum*. *Biofuels*, 11(1): 85-91. https://doi.org/10.1080/17597269.2017.1345357

39. Patel, G. & Shah, K. 2020. Isolation, screening and identification of Lipase producing fungi from cotton seed soapstock. *Indian Journal of Science and Technology*, 13: 3762-3771.

40. Pavlukova, E., Belozersky, M. & Dunaevsky, Y. 1998. Extracellular proteolytic enzymes of filamentous fungi. *Biochemistry. Biokhimiia*, 63(8): 899-928.

41. Pérez, M.M., Gonçalves, E.C.S., Vici, A.C., Salgado, J.C.S. & de Moraes Polizeli, M.d.L.T. 2019. Fungal lipases: versatile tools for white biotechnology, pp. 361-404. In: Yadav, A.N., Mishra, S., Singh, S. & Gupta, A. (eds). *Recent Advancement in White Biotechnology Through Fungi*, Springer, 528 pp.

42. Qader, M.M., Hamed, A.A., Soldatou, S., Abdelraof, M., Elawady, M.E., Hassane, A.S., Belbahri, L., Ebel, R. & Rateb, M.E. 2021. Antimicrobial and antibiofilm activities of the fungal metabolites isolated from the marine endophytes *Epicoccum nigrum* M13 and *Alternaria alternata* 13A. *Marine Drugs*, 19(4): 232. https://doi.org/10.3390/md19040232

43. Raghav, D., Jyoti, A., Siddiqui, A.J. & Saxena, J. 2022. Plant-associated endophytic fungi as potential bio-factories for extracellular enzymes: Progress, Challenges and Strain improvement with precision approaches. *Journal of Applied Microbiology*, 133(2): 287-310. https://doi.org/10.1111/jam.15574

44. Romano, I., Vitiello, G., Gallucci, N., Di Girolamo, R., Cattaneo, A., Poli, A. & Di Donato, P. 2022. Extremophilic Microorganisms for the Green Synthesis of Antibacterial Nanoparticles. *Microorganisms*, 10(10): 1885.

45. Sabotič, J., Trček, T., Popovič, T. & Brzin, J. 2007. *Basidiomycetes* harbour a hidden treasure of proteolytic diversity. *Journal of Biotechnology*, 128(2): 297-307. https://doi.org/10.1016/j.jbiotec.2006.10.006

46. Salwan, R. & Sharma, V. 2020. Molecular and biotechnological aspects of secondary metabolites in actinobacteria. *Microbiological Research*, 231: 126374.

47. Sánchez-Moreno, C., Larrauri, J.A. & Saura-Calixto, F. 1998. A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food Agriculture Ecosystems & Environment*, 76(2): 270-276.

48. Sikandar, A., Zhang, M., Wang, Y., Zhu, X., Liu, X., Fan, H., Xuan, Y., Chen, L. & Duan, Y. 2020. Mycochemical screening and analysis, antioxidant activity, and biochemical composition of fermentation strain Snef1216 (*Penicillium chrysogenum*). *Journal of Analytical Methods in Chemistry*, 1-8. https://doi.org/10.1155/2020/3073906

49. Sohail, M., Ahmad, A. & Khan, S.A. 2016. Production of cellulase from *Aspergillus terreus* MS105 on crude and commercially purified substrates. *3 Biotech*, 6(1): 1-8.

50. Souza, M.T.S., Marinho, B.M., Pasin, T.M., Nelson, D.L. & Benassi, V.M. 2020. Prospection of Filamentous Fungi and the Production of Amylase by *Aspergillus sp*. M1. 7.2. *The Journal of Engineering and Exact Sciences*, 6(3): 0365-0376.

51. Srikar, S.K., Giri, D.D., Pal, D.B., Mishra, P.K. & Upadhyay, S.N. 2016. Green synthesis of silver nanoparticles: a review. *Green Sustainable Chemistry*, 6(1): 34-56. https://doi.org/10.4236/gsc.2016.61004

52. Sun, Y., Qian, Y., Zhang, J., Wang, Y., Li, X., Zhang, W., Wang, L., Liu, H. & Zhong, Y. 2021. Extracellular protease production regulated by nitrogen and carbon sources in *Trichoderma reesei*. *Journal of Basic Microbiology*, 61(2): 122-132.

53. Topal, Ş., Pembeci, C., Borcaklı, M., Batum, M. & Çeltik, Ö. 2000. Türkiye'nin tarımsal mikoflorasının endüstriyel öneme sahip bazı enzimatik aktivitelerinin incelenmesi-I: Amilaz, proteaz, lipaz. *Turkish Journal of Biology*, 24: 79-93.

54. Vansteelandt, M., Kerzaon, I., Blanchet, E., Tankoua, O.F., Du Pont, T.R., Joubert, Y., Monteau, F., Le Bizec, B., Frisvad, J.C., Pouchus, Y.F. & Grovel, O. 2012. Patulin and secondary metabolite production by marine-derived *Penicillium* strains. *Fungal Biology*, 116(9): 954-961. https://doi.org/10.1016/j.funbio.2012.06.005

55. Vázquez-Montoya, E.L., Castro-Ochoa, L.D., Maldonado-Mendoza, I.E., Luna-Suárez, S. & Castro-Martínez, C. 2020. Moringa straw as cellulase production inducer and cellulolytic fungi source. *Revista Argentina De Microbiologia*, 52(1): 4-12.

56. Ventosa, A. 2004. *Halophilic microorganisms*. Berlin, Germany: Springer, 350 pp.

57. Verma, V.C., Kharwar, R.N. & Gange, A.C. 2010. Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. *Nanomedicine*, 5(1): 33-40. https://doi.org/10.2217/nnm.09.77

58. Wadia, T. & Jain, S.K. 2017. Isolation, screening and identification of lipase producing fungi from oil contaminated soil of Shani Mandir Ujjain. *International Journal of Current Microbiology and Applied Sciences*, 6(7): 1872-1878.

59. Wei, Y. & Zhang, S.-H. 2018. Abiostress resistance and cellulose degradation abilities of haloalkaliphilic fungi: applications for saline–alkaline remediation. *Extremophiles*, 22: 155-164.

60. Wei, Y. & Zhang, S.-H. 2019. Haloalkaliphilic Fungi and Their Roles in the Treatment of Saline-Alkali Soil, pp. 535-557. In Tiquia-Arashiro, S. & Grube, M. (eds.). *Fungi in Extreme Environments: Ecological Role and Biotechnological Significance*, Springer, XVIII, 626 pp.

61. Yike, I. 2011. Fungal proteases and their pathophysiological effects. *Mycopathologia*, 171(5): 299-323.

62. Zhang, X., Li, S.-J., Li, J.-J., Liang, Z.-Z. & Zhao, C.-Q. 2018. Novel natural products from extremophilic fungi. *Marine Drugs*, 16(6): 194. https://doi.org/10.3390/md16060194