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Research Article

ISOLATION OF A NOVEL ANTIMICROBIAL POLYPEPTIDE FROM AN *Aspergillus niger* ISOLATE

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Abstract: In this study the extracellular proteins from the isolate LC3 belonging to *Aspergillus* were purified for new antimicrobial polypeptide (AMP) discovery and then tested for antimicrobial activity against *Staphylococcus aureus* (ATCC 25923) and Methicillin-resistant *S. aureus* (MRSA). Antimicrobial activity was determined by the trypsin/proteinase K assay, which was polypeptide-based, and it was observed that this protein was a protein of about 11 kDa by gel overlay assay. The minimum inhibitory concentration of purified AMP molecule against *S. aureus* ATCC 25923 and MRSA was 8 µg/ml and 32 µg/ml, respectively and the AMP molecule was confirmed. ITS sequence analysis showed that isolate LC3 was identified as *Aspergillus niger*, using the Bioedit sequence assembly program. The sequence was deposited with the GenBank database with accession number MK332597. The results indicate that the purified AMP molecule has the potential to be used in infections caused by *S. aureus*.

Özet: Bu çalışmada *Aspergillus*'a ait izolat LC3'ün ekstrasellüler proteinleri, yeni antimikrobiyal polipeptit (AMP) keşfi için saflaştırıldı ve ardından *Staphylococcus aureus* (ATCC 25923) ve Metisiline dirençli *S. aureus*'a (MRSA) karşı antimikrobiyal aktivite açısından test edildi. Antimikrobiyal aktivitenin polipeptit kaynaklı olduğu tripsin/proteinaz K testi ile belirlendi ve bu proteinin jel overlay testi ile yaklaşık 11 kDa'lık bir protein olduğu gözlendi. Saflaştırılmış AMP molekülünün *S. aureus* ATCC 25923 ve MRSA'ya karşı minimum inhibisyon konsantrasyonu sırasıyla 8 µg/ml ve 32 µg/ml'dir ve AMP molekülü doğrulandı. ITS sekans analizi, LC3 izolatının Bioedit sekans birleştirme programı kullanılarak *Aspergillus niger* olarak tanımlandığını gösterdi, sekans, MK332597 erişim numarasıyla GenBank veri tabanına kaydedildi. Bu sonuçlar, saflaştırılmış AMP molekülünün *S. aureus*'un neden olduğu enfeksiyonlarda kullanılma potansiyeline sahip olduğunu göstermektedir.

Introduction

Antibiotics have long been used in treatment of infectious diseases since the demonstration of inhibitory effect of penicillin on microorganisms. One of the main problems in antibiotic use is the loss of their effectiveness over time as a result of the resistance gained by the target microorganisms by various mechanisms (Alanis 2005, Frieri *et al.* 2017). The emergence of antibiotic-resistant microorganisms and increasing concerns about the use of antibiotics have led to the development of antimicrobial peptides (AMPs), which have effective application prospects in medicine, food, husbandry, agriculture and aquaculture. AMPs are not affected by many antibiotic resistance mechanisms that prevent antibiotic use (Apan 2004, Huan *et al*. 2020). AMPs do not induce widespread resistance due to cell membrane specificity and effects on specific protein targets. Due to these properties, AMPs are attracting attention as a source of new antimicrobial agents (Zasloff 2002, Yazici *et al.* 2018).

AMPs are small polypeptides that are important members of the natural immune system and have 12-100 amino acids synthesized by various organisms, usually with broad-spectrum microbicidal activity (Hancock & Diamond 2000). Researches have shown that AMPs do not only have antimicrobial but also have immunomodulator, apoptotic, anticancer and anti-inflammatory properties (Hancock & Diamond 2000, Bachère *et al.* 2004). Although AMPs are generally cationic, there are also anionic ones that contain a few acidic amino acids such as aspartic acid and glutamic acid (Bachère *et al.* 2004, Jenssen *et al.* 2006). They are evolutionarily protected molecules found in many organisms, from prokaryotes to humans. The therapeutic

applications of AMPs are still limited due to the high cost of production, enzymatic degradation and short half-life. They also show enormous toxic side effects on mammalian cells in long-term use (Luong *et al*. 2020). Researchers focused on biological resources to overcome these limitations and discover new antimicrobial resources. New biological discoveries for AMPs will pave the way for in silico approaches in terms of providing new gene resources (Jenssen *et al*. 2006, Huan *et al*. 2020). Lysozyme, the first reported human AMP, was described in 1922 by Alexander Fleming from the nasal mucus (Nizet *et al*. 2001), and since then a total of 3,324 AMPs have been identified in the Antimicrobial Polypeptide Database (ADP3) (https://aps.unmc.edu/). Among these, over 2,500 polypeptides belong to mammals, plants, and bacteria. On the other hand, the number of fungal derived AMPs is 25 of which only 3 belong to members of the genus *Aspergillus* (https://aps.unmc.edu/AP/). Filamentous fungal species found in nature produce metabolites of industrially and medically importance, such as antibiotics and AMPs. The antimicrobial agents, which are secondary metabolites, control bacterial infections and are used in various pharmaceutical processes (Zjawiony 2004). Researchers have shown that compounds produced by various fungi have approximately 126 different therapeutic functions (Wasser 2011). The majority of fungi varieties have been found to have antibacterial, antiviral, antioxidant, anti-inflammatory, antitumor and immune modulation effects (Smith *et al.* 2003), showing that fungi are a potential source to be used for discovery of new AMPs. Altough not much in number, studies showed that the fungal species *Aspergillus niger* have antimicrobial properties. In another study, *in vitro* antibacterial and antifungal activity of *A. niger* (MTCC-961) extract was screened against *Aspergilus flavus, A. fumigatus, Bacillus coagulans, B. licheniformis, Corynibacterium glutamicum* and *Staphylocuccus aureus* and the results showed that *A. niger* have antifungal and antibacterial properties against the used microorganisms (Kalyani & Hemalatha 2017).

In the present study, the LC3 isolate of the filamentous fungus Aspergillus *niger* was chosen as the test microorganism and was investigated for purification of a novel AMP with the potential to be used agains *S. aureus* and Methicillin-resistant *S. aureus* MRSA, both causing clinical infections worldwide (Tong *et al*. 2015). The results revealed a potent antimicrobial effect of a polypeptide of about 11 kDa purified from the isolate against the two microorganism tested.

Materials and Methods

Microorganisms and their maintenance

The isolate LC3 found to have antimicrobial properties in a previous study (Yazici *et al*. 2021) was investigated for new AMP production. The isolate LC3 was incubated for 7 days at 25°C by shaking at 150 rpm in yeast-peptone-glucose (YPG) medium. The antimicrobial activity of the culture filtrate obtained from the isolate was confirmed by proteinase K/trypsin treatment, which was polypeptide-based. *Staphylococcus aureus* ATCC 25923 (clinical isolate) and Methicillin-resistant *S. aureus* (MRSA) (clinical isolate) were used as the reference pathogens and maintained in Mueller Hinton agar (MHA) at 37°C.

The cell-free supernatants (CFS) were prepared for use in agar diffusion, trypsin/ proteinase K treatment and protein isolation experiments. After 7 days of incubation of the isolate LC3 in a YPG medium, the mycelium was filtered with four cheesecloth layers, then centrifuged for 10 min at 9,000 rpm. The supernatant was passed through a 0.22 µm syringe filter and stored at +4°C for further analysis (Gun Lee *et al.* 1999).

Determination of Antibacterial Effect of the Isolate LC3

Agar Diffusion Assay

Agar diffusion assay was used to determine the antimicrobial activity of the isolate LC3. In the agar diffusion assay, a soft Mueller Hinton broth (MHB) with 0.8% agar added was prepared by the pour plate method. Wells were punched out with a 6 mm cork borer and 200 µl of culture filtrates were added to these wells. The Petri plate was incubated at 30°C for 24 hours. At the end of the period, inhibition zone diameters were measured *S. aureus* ATCC 25923 and MRSA (Wiegand *et al*. 2008).

Cross Streak Assay

The isolate LC3 was first grown in YPG agar medium at 25° C for 7 days. On the 7^{th}-day, bacteria strains were inoculated by a single streak of inoculum to stay 1 cm to the fungus colony zone. The growth of bacteria on day 8 was evaluated (Lertcanawanichakul & Sawangnop 2011).

Trypsin/Proteinase K Digestion Assay

Trypsin/Proteinase K Digestion Assay was applied by modifying the assay used in Yazici *et al* (2021). The culture filtrate of the isolate LC3 was incubated with trypsin/proteinase K enzymes at 37°C for 6 hours to find out whether the zones formed in the agar diffusion test were polypeptide-based and at 100°C for 5 minutes to degrade the enzymes. In addition to the protease treatment, the CFS was incubated at 100°C for 5 minutes and non-treatment CFS was used as a control. At the end of the incubation, the results of trypsin/proteinase K Digestion Assay were determined by agar diffusion assay (Yazici *et al*. 2021).

Protein isolation and gel overlay assay

The ultrafiltration was performed to concentrate the culture filtrates obtained from the liquid culture of the isolate LC3. 60 ml of CFS was added to a 3 kDa cut-off ultrafiltration column and concentrated. For total protein isolation, concentrated CFS was mixed with an equal volume of 20% trichloroacetic acid (TCA) by pipetting and incubated at -20°C for 2 hours. At the end of the period, the mixture was precipitated by centrifugation at 13,500 rpm for 10 minutes. It was washed 3 times with 70% ethanol and centrifuged at 13,500 rpm for 5 minutes. Finally, after washing with acetone, the pellet was dried

at room temperature and stored at -20°C (Chen *et al*. 2005). Total protein was determined by the Bradford method (Kruger 2009).

The ultrafiltration process was repeated for fermenter culture for 7 days for protein isolation and antimicrobial assays. Protein samples were run at 80 V for 3 hours in Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). When the running was complete, the gel was removed from the cassette and stained with Coomassie R-250 for 20 minutes. It was left in the wash solution for 1 hour to display the proteins. The gel was photographed with the Bio-Rad gel imaging system (Schägger 2006). To confirm the antimicrobial activity and to determine which protein band is responsible for this activity, a gel overlay assay was used. For gel overlay assay, the CFS sample was run at 150 V for 1 hour in native-PAGE (non-denaturing electrophoresis). The gel was then divided into two parts after electrophoresis. The first gel was used for the image, and the second gel was used for the gel overlay assay. The gel overlay assay was applied by modifying the assay of Valore *et al*. (1996).

Briefly, a poor agarose (0.03% TSB in 0.01 M sodium phosphate, pH; 7.4, with 1% agarose) with low sodium concentration was mixed with the pathogen to make up the bottom layer. A piece of the native-PAGE gel was placed on top at 37°C for 3 hours for proteins to diffuse from the gel onto the poor agarose. The gel was then removed, and a piece of poor agarose containing the gel was cut. Poor agarose piece was placed on a rich agarose (6% TSB in 0.01 M sodium phosphate, pH; 7.4, 1% agarose).

As a result of overnight incubation, the protein corresponding to the clear zone was found to be responsible for the antibacterial activity (Valore *et al*. 1996).

Purification of AMP

The protein found to be responsible for antimicrobial activity was centrifuged at 13,500 rpm for 10-15 minutes using the appropriate cut-off ultrafiltration column (30 kDa). Then, the protein sample was stored at -20°C for MIC determination.

Evaluation of Production of AMPs According to Days

For the evaluation of AMP production according to days and the effect of physiological conditions on AMP production, fermenter production was carried out after flask culture. For this, inoculated 10 ml of the reference culture with fungi grown on 7 day a YPG medium to the 3-liter fermenter. The isolate LC3 was grown according to the parameters pH, temperature, shake speed and dissolved oxygen level; 6.2, 25°C, 300 rpm, %10±2, respectively in the fermenter. Agar diffusion, protein isolation and SDS-PAGE assay were performed according to previously described protocols.

Determination of Minimum Inhibitory Concentration (MIC)

To determine the Minimum Inhibitory Concentration **(**MIC) value of the polypeptide isolated from the isolate LC3, the protocols received from the European Committee for Antimicrobial Susceptibility Testing (EUCAST) have been applied (http://www.eucast.org/). MIC is the smallest amount of concentration that prevents the development of microorganisms.

A broth microdilution method was performed to determine MIC value against *S. aureus* and MRSA. Briefly, 100 µl of bacterial cells at a concentration of 0.5 McFarland and protein isolated at different concentrations (0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 µg/ml) were added in in 96-well plates. The total volume was completed to 200 µl with MHB. The plates were incubated at 37°C for 24 h. At the end of the period, MICs were determined.

Identification of Antimicrobial Peptide Producer Isolate

DNA isolation was performed manually from a 7-day solid culture with the phenol-chloroform assay. 1 ml of lysis buffer was added to a sample of mycelium taken into 2 ml Eppendorf tube. The tube was placed in a homogenizer at 12,000 rpm for 10 minutes. After centrifugation, the supernatant was taken up in a new tube and 2 µl of RNase A was added. The mixture was incubated at 37°C for 15 minutes. 1 volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added to the mixture. It was vortexed and centrifuged at 12,000 rpm for 10 minutes. The upper part was taken into a new tube and the DNA was precipitated with 100% ethyl alcohol at -20°C for 1 hour. After centrifugation at 12,000 rpm for 10 minutes, the pellet was washed three times with 70% ethyl alcohol and dried in 50 µl TE buffer (Sambrook & Russell 2006).

Amplification of the ITS Region was performed with PCR to make the molecular identification. ITS 1 primer (5 'TCC GTT GGT GAA CCA GCG G 3') and ITS 4 primer (5 'TCC TCC GCT TAT TGA TAT GC 3') were used to amplify the 18S-ITS1-5.8S-ITS2-28S region. The PCR conditions included; initial denaturation (95°C for 2 min); 35 cycles [95°C for 45 sec, 55°C for 45 sec, 72°C for 1 min] and a final extension at 72°C for 10 min. The amplicons were sequenced using ITS1 and ITS4 primers. Then, the Bioedit sequence assembly program was used and submitted to the BLASTN 2.13.0+ (Zhang *et al*. 2000).

Results

Determination of antimicrobial activity

Agar Diffusion Assay

The results of the Agar Diffusion Assay showed that the culture filtrate of the isolate LC3 has an antimicrobial effect against *S. aureus* (ATCC 25923) and MRSA.

Table 1. The results of Agar Diffusion Assay with flask and fermenter cultures.

	Staphylococcus aureus (ATCC 25923) (mm)				MRSA (mm)			
	4th day	5th day	6th day	7th day	4th day	5th day	6th day	7th day
Flask culture	22±1.5	23±0.8	23±1.7	24±0.9	22±1	23±0.5	23±1.3	23±1.2
Fermenter culture	-	-	14±0.7	15±1.3	-	-	14±1.2	14±1.4

Note: Results were represented as mean ± SD, n = 3.

Cross Streak Assay

The cross-streak assay was performed to determine whether microorganisms have antagonistic effects against each other (Lertcanawanichakul & Sawangnop 2011). The isolate LC3, *S. aureus* and MRSA bacteria were cultured on YPG agar medium. After 1 day of incubation, the absence of *S. aureus* and MRSA growth confirmed the antimicrobial activity of the isolate LC3 against these bacteria. Fig. 1 shows the Petri plate image of the cross-streak assay.

Trypsin/Proteinase K Digestion Assay

The antimicrobial activity in tems of inhibition zones seen in Fig.v2a and Fig. 2c indicates that CFS was not affected by temperature and temperature treatment was not affected by the protease treatment assay result. The loss of antimicrobial activity in Fig. 2b showed the antimicrobial compound to be a polypeptide.

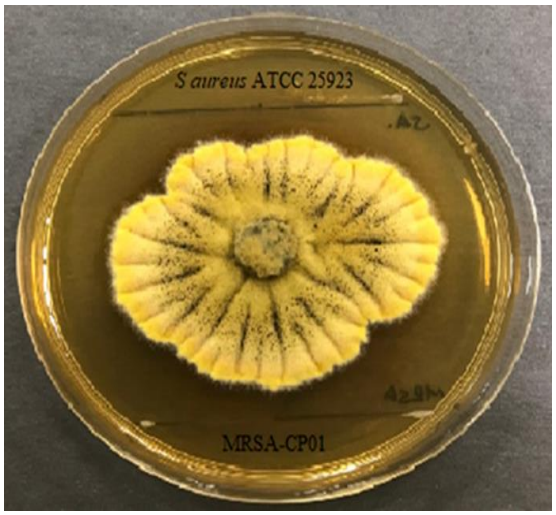

Fig. 1. The appearance of the Petri Plate used in the Cross Streak Assay against *S. aureus* and MRSA.

Protein Isolation and Gel Overlay Assay

According to the results given in Fig. 3, the total proteins of the fungus grown in flask culture gave the most intense band in the region of about 11 kDa.

The SDS-PAGE was performed for gel overlay assay and antimicrobial activity was determined using a prepared pour plate method with soft MHB, but as a result of the experiment, the antimicrobial activity could not be determined. Since the clear zone seen in the assay applied according to the gel overlay protocol was not clear, it could not be determined which protein band was responsible for the antimicrobial activity (results not shown) (Liu *et al*. 2012).

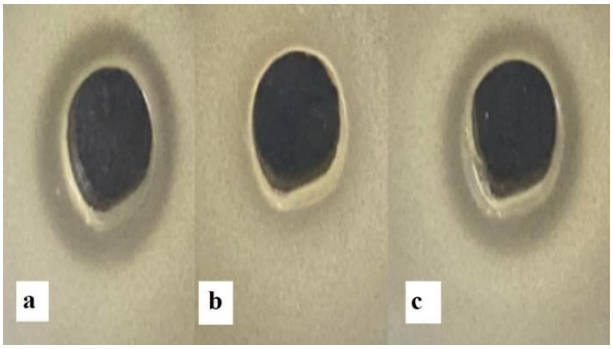

Fig. 2. Agar Diffusion Assay Image of the isolate LC3. **a.** Culture filtrate, **b.** protease treated culture filtrate, **c.** incubation at 100°C Culture Filtrate

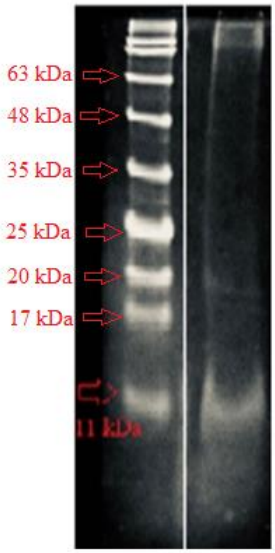

Fig. 3. SDS-PAGE Profile of Extracellular Proteins of the isolate LC3

Proteins are separated according to their native structure in native-PAGE assay and the medium with agarose facilitated the diffusion of proteins in the gel into the medium.

As a result of modifications to the assay, the native-PAGE gel detected the protein band responsible for antimicrobial activity. This experiment contributes to determination of our AMP.

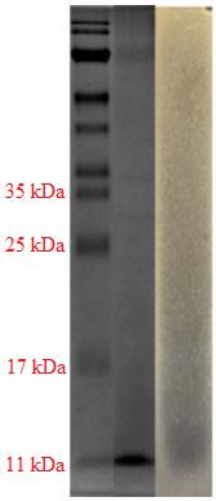

Fig. 4. The Gel Overlay of CFS against *S. aureus*

According to band profiles in Fig. 4, the gel overlay assay showed zones of inhibition against *S. aureus* starting from about 11 kDa. According to these results, the band showing antibacterial activity is a protein of about 11 kDa.

Purification of AMP

The antimicrobial polypeptide was determined with Native-PAGE and gel overlay assay. The polypeptide was then run with SDS-PAGE, which was purified with a 30 kDa cut-off ultrafiltration column (Fig. 5). This purified polypeptide was used for the determination of the MIC value.

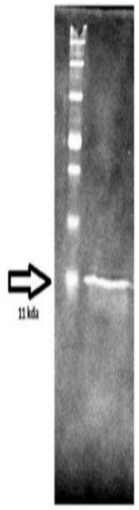

Fig. 5. Crude Protein Band Showing Antimicrobial Activity.

Evaluation of Production of AMP According to Days

The total protein production of fungus grown in fermenter culture and antimicrobial activity of CFSs were determined according to days. SDS-PAGE of the total proteins of the fungus according to the protein band profile is shown in Fig. 6a. The antimicrobial activity of the fermenter culture according to days is shown in Fig. 6b. The protein band, about 11 kDa, which was responsible for antimicrobial activity (Fig. 6a) began to appear on the 5th day, but the antimicrobial activity in the agar diffusion assay appeared on the 6th day (Fig. 6b). The absence of this activity on the 5th day may be due to the fact that the about 11 kDa band responsible for the antimicrobial activity is less dense than on the other days.

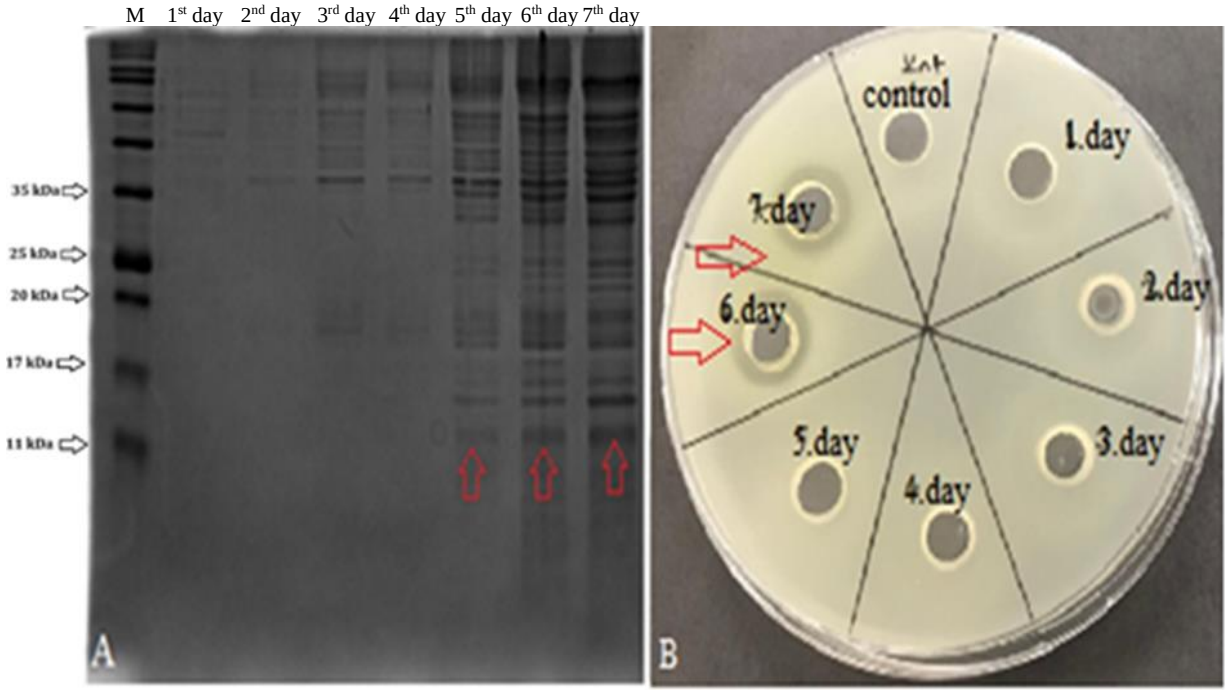

Fig. 6. a. Total proteins of the isolate LC3 grown in fermenter culture, **b.** agar diffusion results.

Determination of MIC Value

The MIC values of the purified AMP molecules against *S. aureus* 25923 and MRSA were 8 µg/ml and 32 µg/ml, respectively.

Identification of the Antimicrobial Peptide Producer Isolate

Molecular characterization has been done for the isolate LC3. rDNA sequence data was interpreted using BLASTN 2.13.0+ and Bioedit program, then isolate LC3 was identified as *Aspergillus niger* with accession number MK332597.

Discussion

Aspergillus niger has biotechnological importance as it produces various enzymes, organic acids and removes waste materials. Citric acid and many *A. niger* enzymes are recognized as generally recognized as safe (GRAS) by the United States Food and Drug Administration. *Aspergillus* species have received great attention as sources of new antimicrobial agents (Al-Fakih & Almaqtri 2019).

In one of the few studies showing that *A. niger* has antimicrobial properties, Subhash *et al*. (2022) evaluated the antibacterial activity of *A. niger* culture filtrate (ACF) against enteric pathogens *Escherichia coli, Klebsiella pneumoniae, K. variicola, Pseudomonas aeruginosa, Salmonella enterica, Shigella dysenteriae, Staphylococcus aureus* and *Vibrio cholerae* and demonstrated that ACF inhibited the growth of these pathogens (Subhash *et al.* 2022). Similarly, in our study, *A. niger* culture filtrate was found to have antimicrobial activity against *S. aureus* and MRSA. Gun Lee *et al.* (1999) reported that a new antifungal peptide (Anafp) was purified from the culture supernatant of *A. niger*. Anafp exhibited strong growth inhibitory activities against the tested filamentous fungi and the yeast strains in the MIC range of 4 to 15 µM. In contrast, Anafp did not show antibacterial activity against *E. coli* and *Bacillus subtilis* even at MIC=50 µM (Gun Lee *et al*. 1999).

Kalyani and Hemalatha (2017) reported that antibacterial and antifungal activity of crude extract of *A. niger (*MTCC-961) (Kalyani and Hemalatha 2017). In our study, the antimicrobial effect of *A. niger* was revealed by agar diffusion, cross streak and MIC test.

Although the antimicrobial effect of culture filtrates obtained from *A. niger* is relatively well reported, purification of AMP has rarely been studied. According to the results of the agar diffusion assay in our study, antimicrobial efficiency differences between the flask and fermenter culturesoccurred probably due to various parameters such as pH and dissolved oxygen level in growth conditions. Although these parameters were controlled in the fermenter culture, the results were not more effective. Fungi and other microorganisms produce metabolites that inhibit the growth of other microorganisms for nutrient competition and habitat in the natural environment (Al-Fakih and Almaqtri 2019; Cesa-Luna *et al.* 2020). The amount of AMP produced may vary, as the need for nutrients and oxygen is limited in the flask culture. Yazici *et al*. (2021) reported that, CFS of the fungus identified as *A. tubingensis* showed a zone larger than 20 mm against *S.aureus* and MRSA (Yazici *et al*. 2021). Kalyani and Hemalatha (2017) evaluated the antimicrobial activity of CFS of *A. niger* (MTCC-961) against various bacterial and fungal species and the inhibition zone value for *S .aureus* (MTCC-3160) was found to be 12 mm. Al-Shaibani *et al*. (2013) determined the inhibitory effect of CFS of *A. niger* obtained from the inflamed eyes of patients against *P. aeruginosa, S. aureus, S. epidermidis* and *Bacillus* sp., which were isolated from patients of microbial keratitis. The results showed that *A. niger* possessed inhibitory effects against *P. aeruginosa, S. aureus, S. epidermidis,* and *Bacillus* sp. with inhibition zones of 15, 25, 30 and 32 mm, respectively (Al-Shaibani *et al*. 2013). Omeike *et al*. (2019) reported that antibacterial activity of *Geotrichum candidum* OMON-1, *Talaromyces pinophilus* OKHAIN-12, and *Penicillium citrinum* PETER-OOA1 fungal extracts were as 32 ± 0.12, 24 ± 0.2, 12 ± 0.17 mm zones of inhibition against *S. aureus*, respectively. The best result from our data is the zone value of 25 mm and it is similar to the values in the literature. The results of the experiment found clear support that the fungus culture filtrates differed in antimicrobial effects against various bacterial strains.

In the study of Park *et al*. (2008), it was determined that the production of bacterial culture at different scales and physiological conditions changed the antimicrobial activity (Park *et al*. 1998). In the study of Liu *et al.* (2012), the antimicrobial activity of Laparaxin produced from *Lactobacillus paracasei* was investigated and it was found that this polypeptide obtained from the fermenter, where pH was fixed at 6, showed less antimicrobial activity compared to the polypeptide obtained from the non-pH control fermenter (Liu *et al*. 2012). Based on these studies, the reason for the differences in our the results of the SDS-band profiles of culture grown in a flask and fermenter culture can be attributed to the different physiological conditions in which they exist.

A MIC is generally considered to be the most basic laboratory measurement of the activity of an antimicrobial agent against a microorganism. Drugs with a lower MIC value are more effective antimicrobial agents, as a lower MIC value indicates that less drug dose is needed to inhibit the growth of the microorganism (Kowalska-Krochmal and Dudek-Wicher 2021). A correlation was observed between molecular weights and antimicrobial activities of AMPs. However, most researchers report an increase in antimicrobial activity with decreasing molecular weight (Sultana *et al.* 2021). MICs of some AMPs such as LL-37(4.4 kDa), indolicidin(1.9 kDa), pexiganan (2.2 kDa) against *S. aureus* ATCC 25923 are 14 µg/ml, 16 µg/ml, 32 µg/ml, respectively (Ebbensgaard *et al*. 2015). Mygind *et al*. (2005) reported that AMP purified from *Pseudoplectania nigrella* and characterized as plectasin (4.3 kDa) gave a MIC of 4-32 µg/mL against

various MRSA strains (Mygind *et al*. 2005). According to the study of Mataraci and Dosler' (2012), indolicidin and nisin (3.3 kDa) cationic polypeptides have MICs of 16 µg/ml, 16 µg/ml against MRSA, respectively (Mataraci and Dosler 2012). Yazici *et al*. (2021) reported that AMP (11 kDa) purified by the species identified as *A. tubingensis* has 32 µg/ml and 128 µg/ml MIC values against *S. aureus* ATCC 25923 and MRSA, respectively. These studies confirm the relation between molecular weights and antimicrobial activities, and their findings indicate that the MIC value of the purified AMP in our study is consistent with with the MIC values of other studies. Omeike *et al*. (2021) reported that an AMP compound characterized as Tripeptide GP-2B produced by *G. candidum* OMON-1 has 8 µg/mL, 32 µg/mL, 32 µg/mL MIC values against *S. aureus* ATCC 25923, *S. aureus* ATCC 6238, and various strains of MRSA, respectively (Omeike *et al*. 2021). In line with previous studies, MICs of purified AMP gave the same values against *S. aureus 25923* and MRSA.

Conclusion

To date, a total of 3324 antimicrobial proteins have been identified in the Antimicrobial Polypeptide Database (ADP3). Although fungi have the 22 molecules of the AMP identified, only three belong to the *Aspergillus* genus. According to these data, as an AMP source, there is a tendency toward *Aspergillus* isolate LC3 in this study. The antimicrobial activity was revealed by the gel overlay assay, where this antimicrobial activity originated from the about 11 kDa protein band. Then this about 11 kDa protein was purified using a 3 kDa and 30 kDa cut-off ultrafiltration column and the MIC values of the crude protein were determined. As a result, revealing the antimicrobial properties of a novel AMP purified from the isolate LC3 provides a source for uncharacterized proteins. Our data indicate a new attractive therapeutic resource that provides a new light on combating infectious diseases.

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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.

The authors confirm that the data supporting the findings of this study are available within the supplementary material of the article.

Author Contributions: Concept: A.Ü., A.Y., S.Ö., Design: A.Ü., A.Y., S.Ö., Execution: A.Ü., A.Y., S.Ö., Material supplying: S.Ö., Data acquisition: A.Ü., A.Y., S.Ö., Data analysis/interpretation: A.Ü., A.Y., S.Ö., Writing: A.Ü., A.Y., S.Ö., Critical revision: A.Ü., A.Y., S.Ö.

Conflict of Interest: The authors have no conflicts of interest to declare.

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