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Potential of keratinolytic fungi isolated from chicken and goat farms soil, North Sumatra

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Abstract

Efforts have been made to develop fungal isolates with the ability to degrade chicken feather waste. The goal of this study was to find and test the ability of keratinolytic fungi isolated from the soil around chicken and goat farms to degrade chicken feather waste. The keratinolytic fungi were preliminarily screened for their proteolytic activity on skim milk agar medium. The study obtained forty six isolates that were characterized based on the fungal colonies color or fungal morphotypes. Eight fungal isolates (A2, A7, A12, A18, A29, A31, K1, and K2) displayed considerable proteolytic activities and total degradation of chicken feather in the fermentation medium or feather meal broth in 10 days. Based on the molecular analysis, the species identity of potential isolates namely K2, and A18 were assigned as *Yunnania carbonaria* and *Penicillium citrinum*.

Keywords – Chicken Feathers Waste – Degradation – ITS Identification – Keratinolytic Fungi

Introduction

Keratinolytic microorganisms are a group of microbes that produce keratinase and range from bacteria, fungi, to actinomycetes (Brandelli et al. 2010, Mamangkey et al. 2020a, b). Investigating the ability of keratinolytic fungi to degrade chicken feather keratin waste has recently been prominent in order to uncover a viable isolation source. Bungsu (2018) had successfully isolated 19 keratinolytic bacteria from three different areas including snake skin, soil around animal skin tanning home industry, and snake captivation waste. Identification was successfully done and found two potential isolates with chicken feathers' waste degradation skill based on 16s rRNA gene bacteria, those are *Enterobacter tobaci* YIM Hb-3 and *Aeromonas media* RM. Mamangkey et al. (2019a) then reported, in the next year, to have successfully found keratinolytic bacteria isolated from crocodile (*Crocodylus porosus*) feces and landfill.

Fungi have previously been used in research on chicken feather waste. Kumar & Kushwaha (2014) used fungi such as *Acremonium strictum, Microsporum gypseum, Chrysosporium indicum, Chrysosporium tropicum, Penicillium griseofulvum, Malbranchea* sp., *Myceliophthora fergusii,* and *Gymnoascus intermedius* to successfully degrade chicken feather waste. Mamangkey et al. (2019b) effectively recovered 18 keratinolytic fungi from two separate locations of crocodile feces obtained from crocodile breeding farm and soil samples namely THB7, FB4, and THB4, which

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were designated as potential keratinolytic fungal strains in the degradation of chicken feather waste.

Keratin content in chicken feathers became an issue which delays the utilization and degradation of the waste. Keratin has 14% of disulfide bonds, which are stable and rigid structures that are resistant to proteolytic enzymes like trypsin, pepsin, and papain in the digestive system (Brandelli 2008, Mazotto et al. 2011). Although keratin compounds are insoluble and difficult to dissolve, some microorganisms can degrade the polymer by using extracellular keratinase (Puastuti 2007).

Experts in microbiology and biotechnology have been interested in fungi that generate keratinase because they grow on low-cost substrates and produce huge amounts of enzymes in culture media, making waste management easier (Anbu et al. 2005, Friedrich et al. 2005). Microbial keratinases can be potentially applied in biotechnological applications since they are specific and prospective keratin degrading enzymes. They also might be used in animal feed, detergents, fertilizer, leather and textile manufacturing, as well as pharmaceutical and biomedical research. (Brandelli 2008, Gupta & Ramnani 2006, Gupta et al. 2013). Several types of keratinases surprisingly also exhibit antibacterial and antioxidant properties (Kumar et al. 2012, Liu et al. 2017, Fontoura et al. 2019, Mamangkey et al. 2020c). Researchers believe that such utility will aid in the selection of new fungi capable of degrading chicken feather from the soil of chicken and goat farms in the Serdang Bedagai District, North Sumatra.

Materials & Methods

Preparation of Chicken Feathers Substrate

Broiler chicken feathers waste was collected from chicken slaughterhouses in traditional market. For the preparation, as the main material, chicken feathers were washed multiple times. Initially they were washed using water and later on continued using a detergent. The feathers were washed again after half an hour, followed by a drying process under the sunlight. The drying process was also continued by using oven that has been modified at the temperature of 60 ^{o}C for 24 hours (Mini et al. 2015).

Keratinolytic Fungi Isolation

Soil samples were collected from the soil around chicken farm in Serbajadi Sub-District and goat farm in Pegajahan Sub-District, Serdang Bedagai. Fungi were isolated using Skim Milk Agar (SMA) media (Widhyastuti & Dewi 2001). Samples that have been collected were then diluted serially (10^{-1}-10^{-6}) using physiological solution (NaCl 0.9%). Isolation was done by using spread plate method in which 0.1 mL solution of each dilution series was inoculated. The colony was incubated for three days at room temperature, with the clear zone generated around it being monitored on a daily basis. Purification of fungi that formed a clear zone around the colony was done on a new SMA medium.

Measurement of Clear Zone Diameter

The proteolytic activities of fungal isolates were measured quantitatively. Cork borer was used to cut mycelia growth of fungal isolates, which were subsequently inoculated into SMA media and incubated for 6 days. On the third and sixth days of incubations, the clear zone and the fungal colony diameter were measured using a digital caliper.

Measurement of Chicken Feathers Degradation by Keratinolytic Fungi

Keratinolytic fungal isolates have the potential to degrade chicken feathers waste using modified Matikevičienė et al. (2009) method. As much as 100 mL of feather meal broth (FMB) media was transferred into Erlenmeyer flasks containing 0.5g sterile chicken feathers waste (remiges dan retrices). Fungal isolates were cut using cork borer number 4 and inoculated on sterile FMB media. Incubation was performed for 10 days (± 28 ^{o}C) using orbital shaker (120 rpm). Fungi were filtered using Whatman filter paper no.42, while the liquid media was centrifuged to separate

chicken feathers waste remaining (pellet) and liquid phase (supernatant). The degradation result in the form of chicken feathers waste was separated from fungal hyphae and dried at the temperature of 80 ºC until it reached constant weight (± 48 hours) in order to know the decrease of dry chicken feathers' waste weight.

The liquid phase (supernatant) produced from the filtration was centrifuged at the velocity of 10,000 rpm for 10 minutes. Supernatants were then transferred to a new centrifuge tube and added with 0.1 M trichloroacetic acid (TCA). The comparison of supernatant and 0.1 M TCA was 2:1. The mixture was then centrifuged again at the velocity of 10,000 rpm for 10 minutes. Supernatant was measured at the wavelength of 280 nm using spectrophotometer UV-Vis to know the amino acid level produced from the chicken feathers waste degradation. Formula used to determine the chicken feathers weight decrease was as follows.

$$\% \text{ Decrease of Chicken Feathers' Weight} = \frac{initial\ dry\ weight - final\ dry\ weight}{final\ dry\ weight} \times 100\%$$

Preparation of Keratinolytic Fungi for Molecular Identification

Keratinolytic fungi were grown on PDA media and incubated for 2 to 3 days at the temperature of 29 ºC. As much as 40g fungal mycelium was harvested and transferred to 600 µL nucleic lysis solution. DNA was then extracted using Mini Kit Promega (According to the Promega Protocol). Fungal mycelium was homogenized through incubation for 15 minutes at 60 ºC using thermomixer and cooled down until it reached the room temperature of 29-30 ºC. As much as 200 µL protein precipitation solution was added and vortexed for 5 seconds. The result was then centrifuged for 3 minutes (13,000 *xg*). Subsequently the supernatant was transferred into new tube containing 600 µL isopropanol, homogenized and centrifuged for 2 minutes (13,000 *xg*). Supernatant was removed again and transferred to a new tube containing 600 µL ethanol 70% and centrifuged again (2 minutes, 13,000 *xg*). It was further left dry to separate the pellet from the supernatant. In addition, 50 µL rehydration solution DNA was added into tube containing the pellet and vortexed for 5 seconds. As much as 0.5 µL RNAse solution was also added and incubated for 15 minutes at the temperature of 37 ºC. DNA was lastly stored in freezer to be used further.

Keratinolytic Fungi DNA Amplification Primer

The amplification of DNA from ITS rDNA region was used in the research conducted by Fu et al. (2012), which have been previously modified. This stage was performed by using ITS1 (TCCGTAGGTGAACCTGCGG) primer and ITS4 (TCCTCCGCTTATTGATATGC) primer. PCR (60 µL) was composed by 30 µL GoTaq, 18 µL nuclease free water, 3 µL ITS 1 primer (pmol/ µL), 3 µL ITS 4 primer (pmol/µL), and 6 µL DNA template. The mix ingredients of PCR reaction were put into 0.2 mL Eppendorf tube. PCR was at predenaturation condition at 95 ºC (5 minutes), for 35 cycles, denaturation condition at 95 ºC (1 minute), annealing at 55 ºC (35 seconds), elongation at 72 ºC (30 seconds) and continued by final extention 72 ºC (7 minutes). The amplification result was visualized through electrophoresis process. The electrophoresis gel was prepared using 1% agarose (1-gram agarose in 100 mL TAE 1X). The amplified DNA was then purified and sequenced commercially (Macrogen Singapore) to know the DNA bases order. The sequence data were compared with the data in GeneBank at The National Center for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool (BLAST) program and confirmed through a phylogenetic tree construction. The accession number for isolate K2 was MW669580 while for isolate A18 was MT032330 in GenBank.

Results

Keratinolytic fungi from chicken and goat farms soil

This research employed 46 isolates of which 26 isolates were from soil around chicken farm, while the other 20 isolates were from soil around goat farm. All the isolates were subjected to

various colony morphological characteristics grown on skim milk agar (SMA) media. Visual documentation of fungal colony morphological characteristics on the seventh day were shown in Fig. 1.

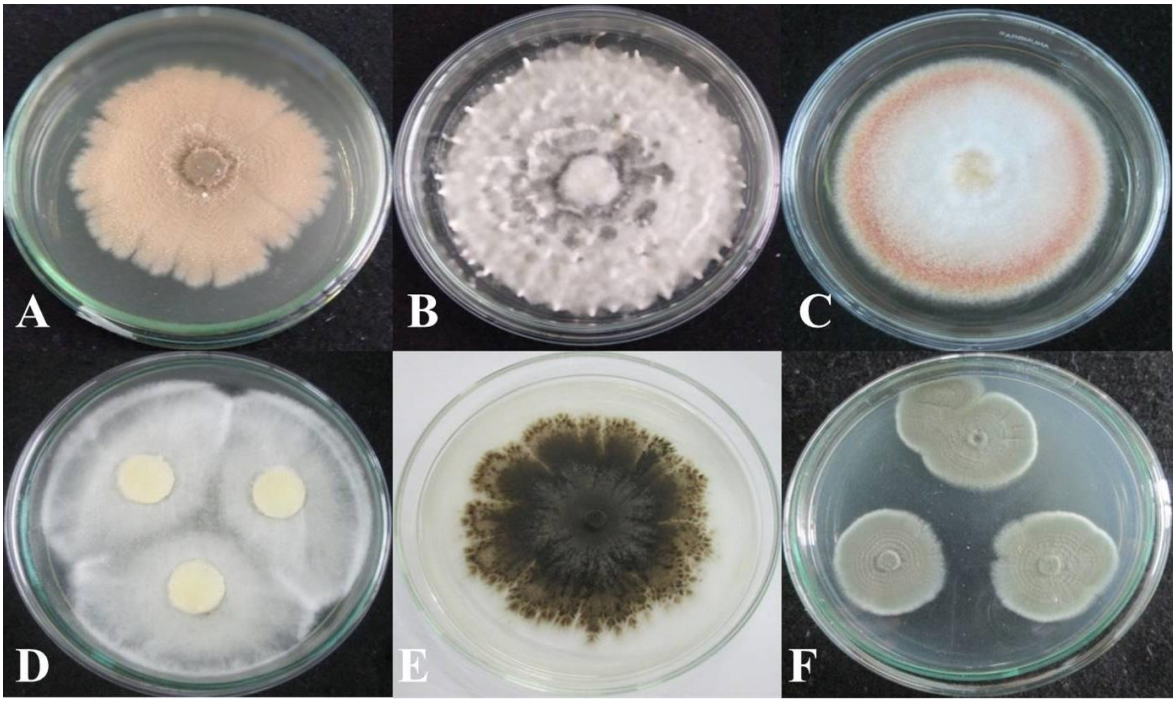

Fig. 1 – Keratinolytic fungal isolate after 7 days incubation. A A8. B A15. C A23. D K21. E K2. F A18.

Measurement of fungal isolates' clear zone

Proteolytic activities of fungal isolates were measured quantitatively (Table 1) and qualitatively (Fig. 2) based on the clear zone produced on SMA media. Clear zone diameter difference indicated the fungal isolates' different potentials in producing protease, especially keratinase. The clear zone of each fungus was measured on the third and sixth day. The following Table 1 presents the data of fungi clear zone measurement results.

Table 1 Clear Zone as Indicator of Proteolytic Activity by Fungal Isolates.

Isolate Code	Clear Zone Diameter (mm)		Fungal Isolates' Diameter (mm)		Isolate Code	Clear Zone Diameter (mm)		Diameter of Fungal Isolates (mm)	
	Day 3	Day 6	Day 3	Day 6		Day 3	Day 6	Day 3	Day 6
A1	27.6	50.95	27.6	50.95	A29	26.7	48.95	24.6	48.95
A2	20.6	30.7	13.95	20.2	A30	34.95	55.4	32.55	51.8
A3	32.75	47	23.3	37	A31	19.8	32.75	11.7	16.9
A5	9.9	25.3	9.9	23.2	K1	29.95	34.25	15.2	22
A7	27.65	46.05	22.9	42.9	K2	26.55	40.1	15.8	26.6
A8	28.65	46.9	26.8	45.8	K3	30.15	47.8	20.15	32.8
A9	46.5	81.65	46.5	81.65	K4	35.4	64.3	34.3	62.8
A10	21.6	36.1	18.95	32.3	K5	22.55	36	13	18
A11	38.4	33.4	55.8	48.25	K6	28.9	42.45	13	20
A12	24.9	35.9	16.75	29.1	K7	41.6	81.3	41.6	81.3
A13	43.7	86.1	43.7	82	K8	25	35.3	13.6	21.5
A15	19.2	35.1	19.2	35.1	K9	36.2	51.5	16.1	28.9
A16	38.35	57.9	31.1	54	K10	25.45	29.9	12.85	26.6

Table 1 Continued.

Isolate Code	Clear Zone Diameter (mm)		Fungal Isolates' Diameter (mm)		Isolate Code	Clear Zone Diameter (mm)		Diameter of Fungal Isolates (mm)	
	Day 3	Day 6	Day 3	Day 6		Day 3	Day 6	Day 3	Day 6
A17	23	39.6	17.55	26	K11	18.1	29.3	16.1	26.9
A18	30.4	48	20.7	32	K13	71	90	65.1	90
A19	16.25	27.25	13.15	23.55	K15	18.1	27.8	16.75	27
A20	23.35	36	19.4	34	K16	32.45	48.65	20.3	33.8
A22	31.5	44.15	25.8	39.95	K17	23.5	36.2	13.9	21.3
A23	40.15	79.75	40.15	79.75	K18	27.85	44.8	17.2	27.9
A24	26.5	50.55	26.5	48.6	K19	45.7	85.3	45.7	85.3
A25	20.15	36.65	16.1	27.65	K21	40.4	71.45	38.5	69.6
A26	29.35	45.75	20.05	32.6	K23	30.65	43.85	19.05	28.35
A28	29	43.4	21.45	29.4	K24	13.1	30.7	11.45	27.6

Furthermore, several fungal isolates' proteolytic activities which have clear zone and qualitatively produced on SMA media are displayes on Fig. 2 below.

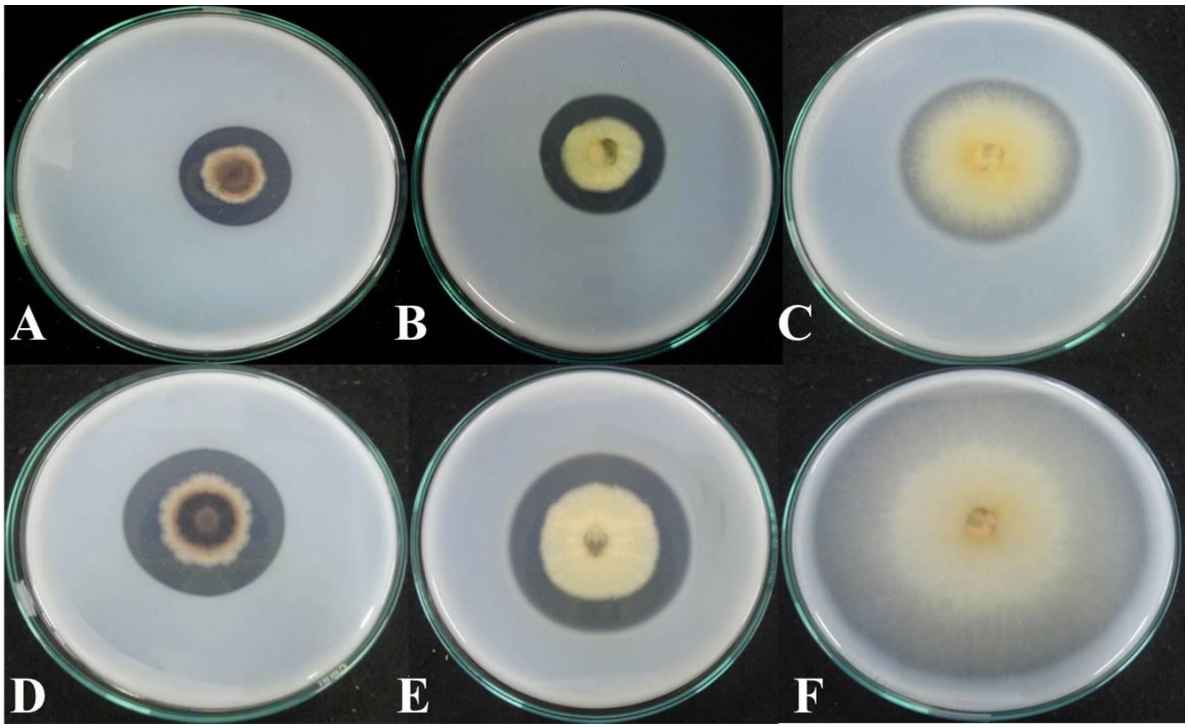

Fig 2 – Fungal Isolates' clear zone. A K2 (Day 3). B A18 (Day 3). C A13 (Day 3). D K2 (Day 6). E A18 (Day 6). F A13 (Day 6).

Determination of Keratinolytic Fungal Strains

Qualitative and Quantitative tests were performed on 46 fungal isolates on SMA media. The following Fig. 3 presents the chicken feathers waste degradation result by keratinolytic fungi using FMB media.

Aerated fungal isolates on FMB media were used to assist the isolates colonize the chicken feather substrates more readily, allowing for greater decomposition. Degradation test result from the overall fungal isolates present various abilities from each different isolates (Table 2).

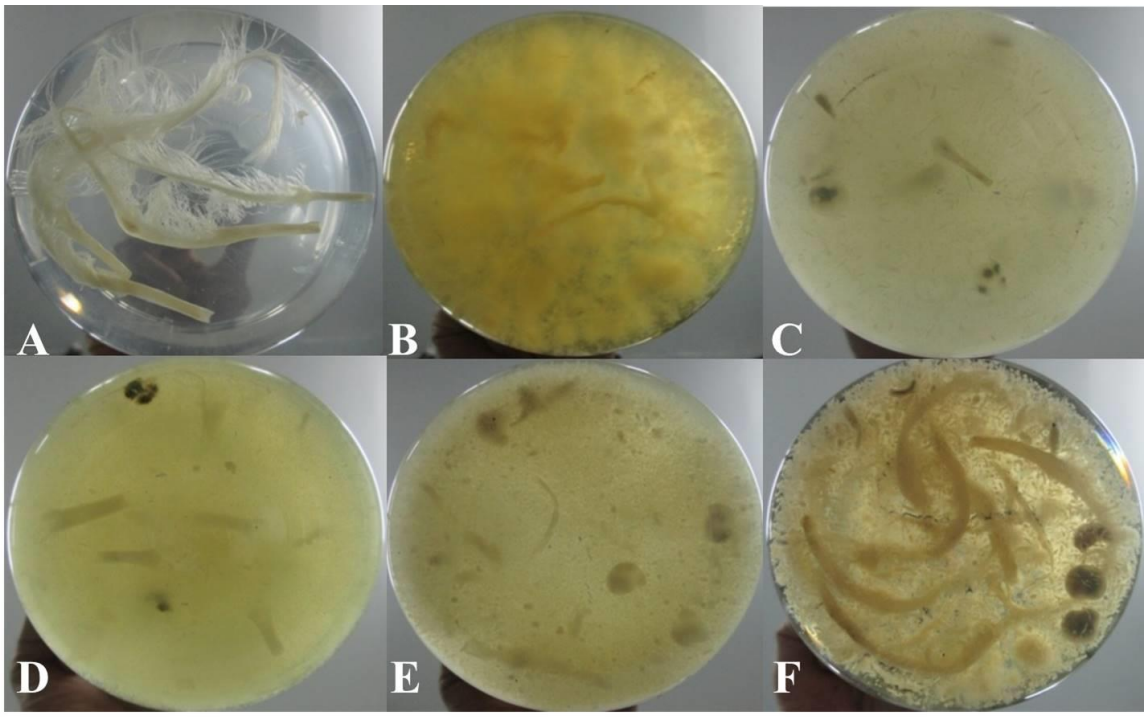

Fig 3 – Biodegradation Test Result of Chicken Feathers Waste after 10 Days Incubation. A Control. B Treatment on Isolate A29. C Treatment on Isolate K18. D Treatment on Isolate A18. E Treatment on Isolate A12. F Treatment on Isolate K2.

Table 2 Chicken Feathers' Final Weight after 10 days Incubation.

Isolates Code	**Final Weight of Feathers (g)**	**% Weight Change**	**Isolates Code**	**Final Weight of Feathers (g)**	**% Weight Change**
	Mean	**Mean**		**Mean**	**Mean**
Control	0.4759	4.82	A30	0.0231	95.38
A1	0.1280	74.40	A31	0	100
A2	0	100	K1	0	100
A3	0.0366	92.68	K2	0	100
A5	0.4428	11.44	K3	0.3585	28.3
A7	0	100	K4	0.0430	91.4
A8	0.2711	45.78	K5	0.0334	93.32
A9	0.0128	97.44	K6	0.0436	91.28
A10	0.0128	97.44	K7	0.0354	92.92
A11	0.0027	99.46	K8	0.4648	7.04
A12	0	100	K9	0.2929	41.42
A13	0.0158	96.84	K10	0.0159	96.82
A15	0.2569	48.62	K11	0.0051	98.98
A16	0.0055	98.90	K13	0.3217	35.66
A17	0.0498	90.04	K15	0.0323	93.54
A18	0	100	K16	0.0029	99.42
A19	0.0255	94.90	K17	0.3524	29.52
A20	0.0121	97.58	K18	0.0022	99.56
A22	0.0428	91.44	K19	0.0011	99.78
A23	0.0121	97.58	K21	0.2124	57.52
A24	0.0304	93.92	K23	0.0503	89.94
A25	0.3952	20.96	K24	0.0229	95.42
A26	0.2454	50.92	A30	0.0231	95.38
A28	0.1920	61.6	A31	0	100
A29	0	100			

Chicken feather waste degradation on FMB media produced amino acids which are contained in the remaining liquid media (filtrate). The addition of 0.1 M TCA in the liquid media served to precipitate the protein so that only amino acids were dissolved in the remaining media. The quantity of amino acids released in the production medium is presented in Table 3.

Table 3 Amount of Amino Acids released.

Isolates Code	Absorbance Value (280 nm) Total Amino Acid	Amino acid tyrosine (mg/mL)	Isolates Code	Absorbance Value (280 nm) Total Amino Acid	Amino acid tyrosine (mg/mL)
	Mean	Mean		Mean	Mean
Control	1.531	233.373	A29	1.577	240.562
A1	1.362	206.968	A30	2.581	397.437
A2	1.841	281.812	A31	1.851	283.375
A3	2.441	375.562	K1	2.212	339.781
A5	1.566	238.843	K2	2.565	394.937
A7	1.678	256.343	K3	1.161	175.562
A8	1.207	182.750	K4	1.765	269.937
A9	2.138	328.218	K5	1.681	256.812
A10	1.063	160.250	K6	1.735	265.250
A11	2.679	412.750	K7	2.598	400.093
A12	2.285	351.187	K8	1.215	184.000
A13	1.551	236.500	K9	1.757	268.687
A15	1.375	209.000	K10	2.191	336.500
A16	2.185	335.562	K11	1.564	238.531
A17	2.071	317.750	K13	1.073	161.812
A18	2.328	357.906	K15	1.659	253.375
A19	1.417	215.562	K16	2.462	378.843
A20	2.062	316.343	K17	1.240	187.906
A22	1.911	292.750	K18	1.881	288.062
A23	1.673	255.562	K19	1.628	248.531
A24	2.398	368.843	K21	2.054	315.093
A25	1.095	165.250	K23	1.903	291.500
A26	1.062	160.093	K24	1.434	218.218
A28	1.283	194.625			

Morphological Characterization and Molecular Identification on Keratinolytic Fungal Isolates

Two potential isolates were chosen based on their abilities in degrading 100% chicken feathers: one was an isolate (A18) collected from the soil around chicken farm (Fig. 4) and another was an isolate collected from goat form which is isolate K2 (Fig. 5).

Genomic DNA electrophoresis results on isolates A18 and K2 visualized on 1% agarose gel is presented in the following Fig. 6.

Genomic DNA amplification through PCR method was conducted by using a pair of universal primers for fungal species identification namely ITS1 primer (TCCGTAGGTGAACCTGCGG) and ITS4 primer (TCCT-CCGCTTATTGATATGC). Genomic DNA of isolate A18 and K2 is successfully amplified after the DNA bands were obtained on 1% agarose gel and 100 kb marker examined under UV rays using Transilluminator. Amplicon of genomic DNA amplification result using PCR method is presented in the following Fig. 7.

DNA 615 bp bands were successfully amplified from isolate A18 and 593 bp from isolate K2. As much as 98.22% identification results of isolate K2 were similar to *Yunnania carbonaria* or

Scopulariopsis members, while isolate A18 had 100% similarities with *Penicillium citrinum* 5110033. The phylogenetic trees of isolates A18 and K2 is shown in the following Fig. 8.

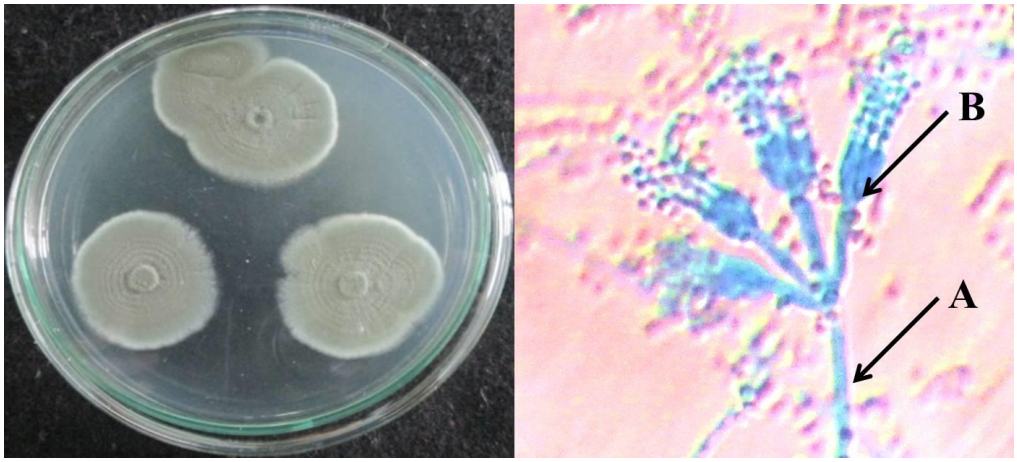

Fig. 4 – Macroscopic and Microscopic Morphology of Fungal Isolate A18. A Conidiophore. B Conidia.

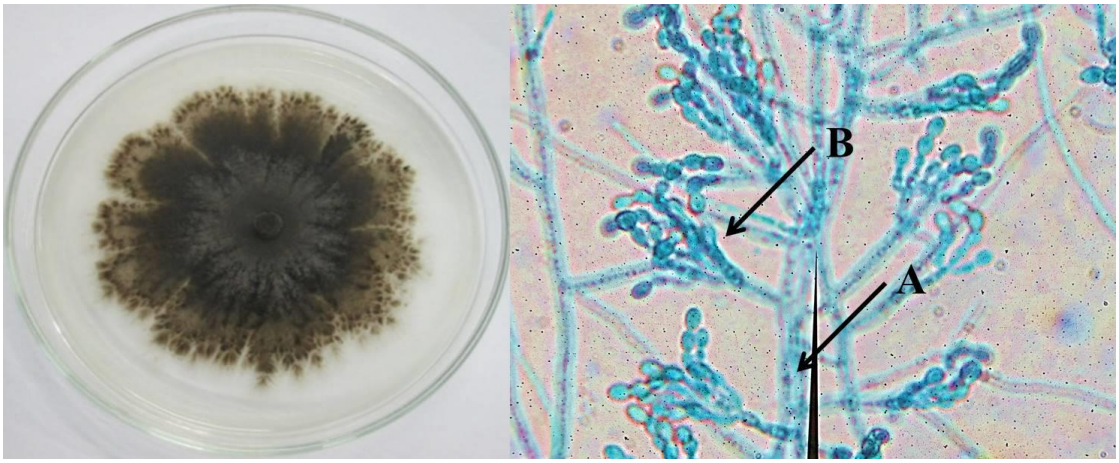

Fig. 5 – Macroscopic and Microscopic Morphology of Fungal Isolate K2. A Conidiophore. B Conidia.

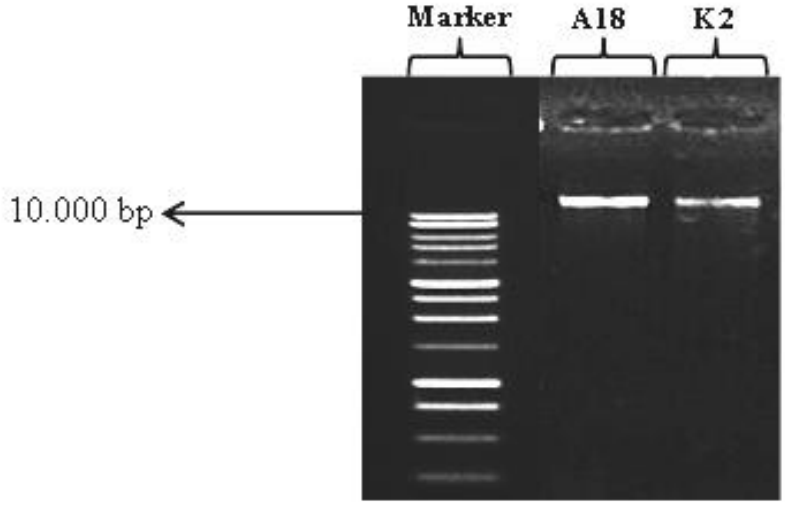

Fig. 6 – Visualization of fungal genomic DNA on agarose gel electrophoresis.

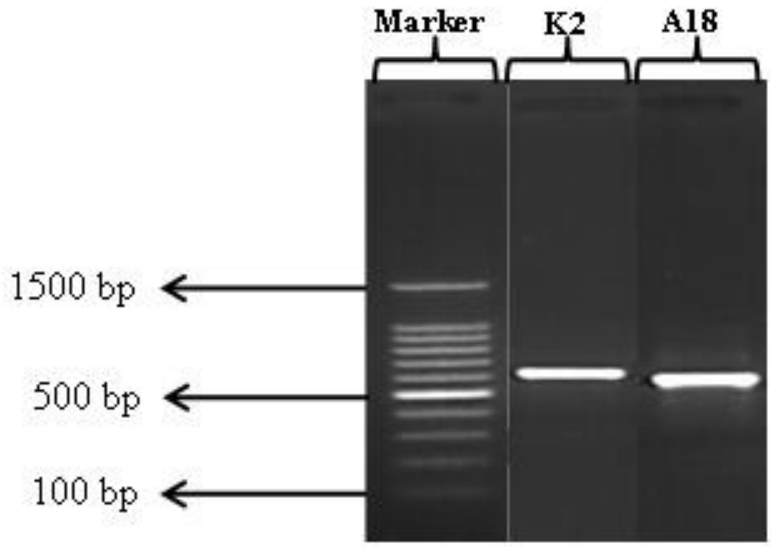

Fig. 7 – Electrogram of Amplification Result on Fungal Isolates K2 and A18.

Discussion

Keratinolytic fungi from chicken and goat farms soil

Because the fungi isolated from the soil around the chicken and goat farms have acclimated to the environment with high keratin, they are capable of producing extracellular proteases. Chicken feathers and goat hair strewn around the soil will cause fungi to release keratinase, which will break down keratin into simpler molecules like amino acids, allowing it to be used as a carbon and energy source. Setyabudi (2015) reported in his research that the growth of fungi on medium with additional casein is an indicator of keratinolytic properties. *Aspergillus niger* degrades keratin substrates by releasing the aromatic amino acid tyrosine into the environment.

There were 26 fungal isolates from chicken farm soil: A1, A2, A3, A5, A7, A8, A9, and A10. Macroscopic observations showed that fungal isolate A1 had a turquoise green top and red bottom, while fungal isolate A2 had a dark gray top and a yellowish white bottom. As many as 20 fungal isolates collected from goat farm soil were obtained: K1, K2, K3, K4, K5, K6, K7, K8, K9, and K10. Macroscopic observation on fungal isolate K1 had a gray top and black bottom, while fungal isolate K2 had a black top and black bottom.

Measurement of Fungal Isolates' Clear Zone

Isolate K13 had the highest proteolytic activity diameter among all fungal isolates tested on SMA media. The clear zone's diameter that can be produced was 71 mm (day 3) and 90 mm (day 6). Isolate A9 also had a large clear zone diameter of 46.5 mm in the third day and 81.65 mm in the sixth day. There was an increase in the fungal isolates' clear zone diameter at the 3rd and 6th day after incubation because each isolate produced different proteases to decompose SMA media. The decomposition of the substrate by the fungi will cause a growth in the fungal colonies' diameter accompanied by a clear zone. The clear zone diameter of several fungal isolates was qualitatively tested on SMA media (Fig. 2).

Based on the data obtained from 46 fungal isolates on the 6th day of observation, about 15.21% of fungi produced clear zones with a diameter of 20 mm to 30 mm, 30.43% of fungi with a diameter of 31 mm to 40 mm, 30.43% fungi with a diameter of 41 mm to 50 mm, 6.52% of fungi with a diameter of 51 mm to 60 mm, 2.17% of fungi with a diameter of 60 mm to 70 mm, 4.43% of fungi with a diameter of 71 mm to 80 mm, and 10.86% of fungi with a diameter of 81 mm to 90 mm. Awasthi & Kushwaha (2011) performed fungal screening test on SMA media isolated from the soil collected in Kampur, India to quantitatively determine the keratinolytic activity of each fungal isolate. A total of 101 fungal isolates were successfully tested and proved to produce clear

zones with a longer incubation period of 8 days. The data obtained that about 23.76% of fungi produce clear zones with a diameter of 21 to 28 mm, 58.41% of fungi with a diameter of 11 to 20 mm, and 17.82% of fungi with a diameter of 2 to 10 mm.

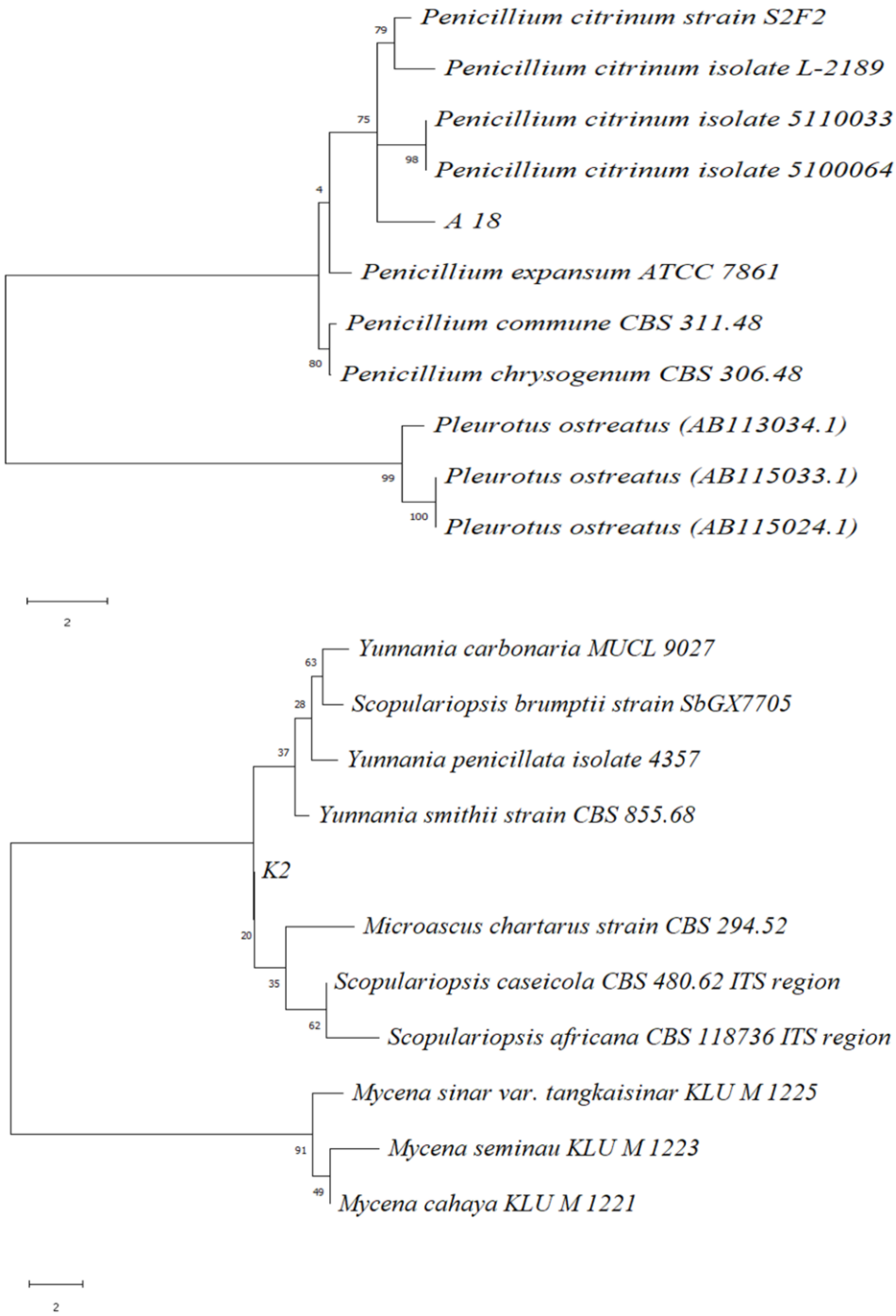

Fig. 8 – Phylogenetic trees of isolates A18 and K2.

Determination of Keratinolytic Fungal Strains

Chicken feathers degradation ability test was performed using fungal isolates on FMB media (Fig. 3) for 10 days incubation. The control treatment did not cause any physical change due to the intact condition of the chicken feathers. The application of fungal isolates to chicken feathers resulted in the colonization of fungal mycelium in the feathers. The presence of a specific enzyme released by fungi, keratinase, within FMB media was considered to be responsible for the degradation of chicken feathers due to the conversion of complex structures to simple compounds in the form of amino acids in the FMB medium.

Eight fungal isolates with isolate codes A2, A7, A12, A18, A29, A31, K1, and K2 had the ability to degrade chicken feathers with 100% changes in chicken feather weight. Research delivered by Calin et al. (2017), tested several fungi such as *Trichophyton* sp., *Fusarium* sp. strain 1A, *Trichoderma* sp., *Cladosporium* sp., *Microsporum* sp., *Fusarium* sp., *Phytophthora* sp., and *Chrysosporium* sp. to find out whether these fungi have potential in degrading horse hair. The degradation test was carried out during a 21 days incubation through shaking. The separation of fungal mycelium from horse hair was carried out by washing it so that the residual degradation test results were obtained and then dried at 60 ^{o}C for 48 hours. *Fusarium* sp. 1A treatment was able to degrade horse hair with a 71.10% change in final weight.

Chicken feathers' waste biodegradation using fungi such as *Aspergillus niger* (Setyabudi 2015); *A. sctritum, C. indicum, C. tropicum* (Kumar & Kuswaha 2014) which had keratinolytic activities was an alternative method that can be employed to increase the keratin waste value from chicken feathers. The structure of keratin protein can be degraded by keratinase produced by microorganisms (Panuju 2003). Keratinase, which is capable of reconstructing the cell wall's chemical network structure, formed the hydrogen and disulfide bonds that make up keratin. (Rodriguez et al. 2009).

Amino acid molecules were detected at a wavelength of 280 nm indicating the degradation of chicken feather waste by fungal isolates. The decomposition of the amino acids resulted from the degradation test of chicken feather waste resulted in the high absorbance value of each fungal isolate. The amino acids that make up keratin include tryptophan, tyrosine and phenylalanine which have aromatic groups. The 280 nm wavelength is a wavelength that has a strong affinity for amino acids that have aromatic rings such as tryptophan, tyrosine and phenylalanine. These amino acids are produced from the breakdown of keratin protein (Tiwary & Gupta 2012).

Molecular Identification of Keratinolytic Fungal Isolates

Quantitative test was done on the molecular identification obtained using a nanofotometer, while the qualitative test was done by conducting electrophoresis. Genomic DNA in isolate A18 had 1.73 purity, while isolate K2 had 1.95 purity. The identification was performed using two primers of ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATGC), which indicated that isolate K2 is *Y. carbonaria* and isolate A18 is *Penicillium citrinum* strain 5110033.

Anbu et al. (2005) have reported that *P. citrinum* has keratinolytic activity in degrading chicken feather waste. *P. citrinum* has been isolated from poultry farm soil in Namakkal and soil around feather waste disposal site in Chennai, India. Whereas, *Yunnania carbonaria* activity has never been reported before. According to Sandoval-denis et al. (2016), *Y. carbonaria* has the characteristics of dark colonies, most of which are brown to brownish green mycelia. Domsch et al. (2007) stated *that Y. carbonaria* can be found in several places such as soil, dust, and air in the room.

According to the result obtained from BLAST, the similarities between fungal isolate A18 to *P. citrinum* strain 5110033 were 100% based on the results of the phylogenetic tree analysis of the fungal isolate A18, which has claded with *P. citrinum* thus it can be concluded that fungal isolate A18 has a very close relationship with *P. citrinum*. *Pleurotus* belonging to Basidiomycota served as the outgroup. The BLAST results of K2 fungal isolate showed 98.22% similarity *with Y. carbonaria* strain CBS 294.52, based on the results of the phylogenetic tree analysis that the K2 isolate showed that the isolate was closely related to the *Yunnania carbonaria*.

Conclusions

- Approximately eight fungal isolates (A2, A7, A12, A18, A29, A31, K1, and K2) are able to degrade the whole chicken feather waste.
- The results of the ITS encoding gene isolate sequences using ITS1 primer (TCCGTAGGTGAACCTGCGG) and ITS4 primer (TCCTCCGCTTATTGATA-TGC) showed that isolate K2 was identified as *Yunnania carbonaria* while isolate A18 was identified as *Penicillium citrinum*.

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