

Phenotypic Characterization of some Actinobacteria and Fungi Isolated From Exposed rock surfaces in Southwestern Sinai, Egypt

Noha Mohamed Kamal^{1,*}, Shimaa Mohamed Abdelsalam¹, Nareman Mosleh Harpy², Maha Amin Hewedy¹ and Ibrahim El-Kattany El-Aassy².

1 Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt. Postal code 11757

2 Geochemical Exploration Department, Nuclear Materials Authority, Qattamia, Cairo, Egypt. Postal code 11936

Abstract:

The search of novel strains continues to be of great importance in research around the world for pharmaceutical, industrial, agricultural and biomining applications. The present study aims to investigating the microbial diversity of rock samples collected from Um Bogma formation, southwestern Sinai, Egypt which was chosen for its unique location, geological and physicochemical properties. The studied samples showed small microbial diversity and low microbial count. A total of ten isolates of actinobacteria and ten isolates of fungi were isolated and characterized phenotypically. The results indicated that all the isolated actinobacteria belong to the genus *Streptomyces*. They were all halotolerant with some showing antimicrobial activities when tested against *Bacillus subtilis*, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Candida albicans* and *Aspergillus flavus*. On the other hand, all the studied fungal isolates belong to the genus *Aspergillus*. They showed resistance to the antifungal nystatin when a concentration of 50 µg per ml was used. The studied locations is characterized by their harsh conditions which does not support the growth of most microorganisms which includes a temperature ranging from below 0°C at night to above 46°C throughout the day, low water content and organic matter hosting radioactive and heavy elements. Rock-dwelling microbial communities that survived such conditions open further

* Corresponding author E-mail address: noha.ghanem@women.asu.edu.eg

research investigations on studying phylogenetic relationships between them as well as their possible microbial activities that can be of environmental and industrial importance.

Keywords: Sinai, rock, actinobacteria, fungi, *Streptomyces*, *Aspergillus*.

1. Introduction

Sinai is a very important and economically-promising region in Egypt due to its unique location, the current developmental projects in it and the various exploitation opportunities it offers. It contains soils and rocks that has been intensively explored and proved to be rich in rare elements, have low organic matter and high heavy metal content (El Galy *et al.*, 2008; Afandy *et al.*, 2010; Hewedy *et al.*, 2013; El-Bialy & Shata, 2018). Its extreme environment is characterized by harsh environmental conditions beyond the optimal range for most microorganisms, which include temperature extremes during the year including frequent summer days, frost days and tropical nights (Abu Bakr *et al.*, 2016; Roushdi *et al.*, 2016). There is few available data on the microbial biodiversity in Sinai rocks and soils, with almost none regarding actinobacteria and fungi (Othman *et al.*, 2003; Hanna *et al.*, 2013).

Microbiota of desert ecosystems plays a role in the biogeochemical cycling of elements as well as balancing the ecosystem (Bhatnagar & Bhatnagar, 2005). Fungi are significant geoactive agents capable of many transformations of metals and minerals that significantly alter the surface structure and chemistry of rocks and their constituent minerals which is important in the bioweathering of rocks as they affect element availability for living organisms (Gadd, 2017). Actinobacteria are widely distributed in different habitats, including soils and rocks. They are involved in important processes and are a source of bioactive compounds (Ghorbani-Nasrabadi *et al.*, 2013). They, especially *Streptomyces*, provides more than half of the naturally occurring antibiotics currently used in pharmaceutical industry (Saadoun & Gharaibeh, 2003; Berdy, 2005; Khandan Dezfully & Gottravalli Ramanayaka, 2015). Also, they are decomposers, digesting many complex compounds, some are nitrogen-fixers, e.g. *Frankia*, produce many enzymes, make nitrification, denitrification as well as other processes that contribute to the biogeochemical cycles (Bhatti *et al.*, 2017).

Accordingly, this study was performed to:

- 1) isolate different rock-dwelling actinobacteria and fungi from rock samples collected from 3 different locations in southwestern Sinai, Egypt.
- 2) phenotypically characterize and tentatively identify the isolated actinobacteria and fungi.

2. Materials and Methods

2.1. Rock samples. Three rock samples were collected from two different locations in Um Bogma environs, East Abu Zenema, southwestern Sinai, Egypt. Samples I and II were collected from Wadi Naseib and sample III was collected from Um Hamd mountain (Fig.1). Samples were placed in sterile container, transferred under aseptic conditions and stored at 4°C until use.

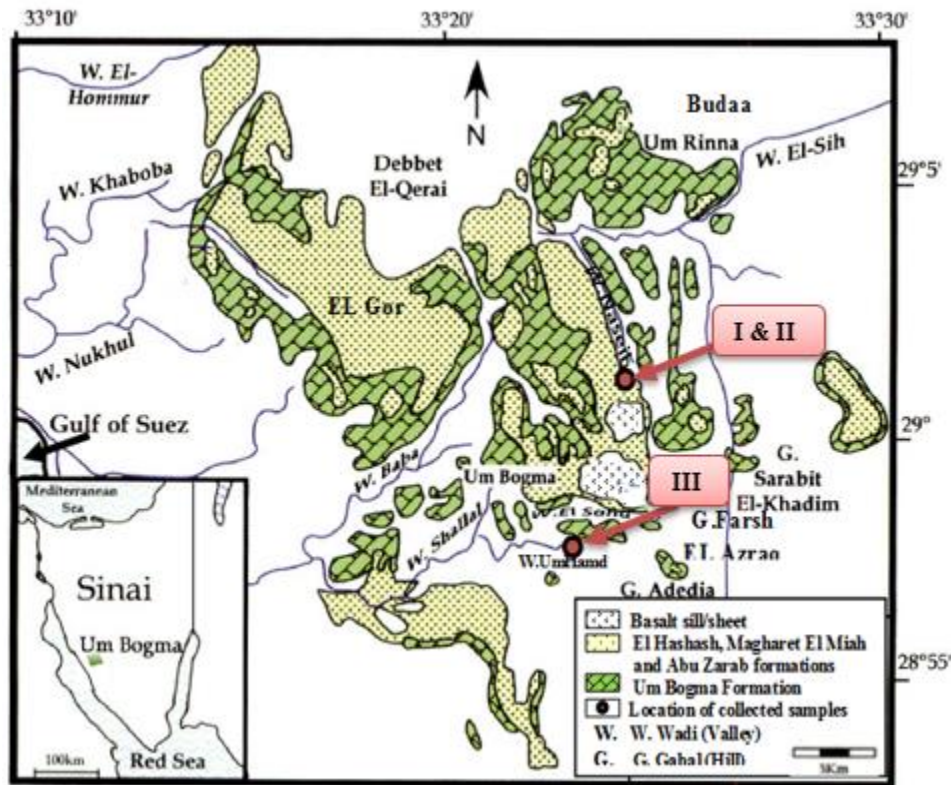


Figure 1: Map of the studied area showing location of the collected samples I, II and III after **Kora and Jux (1986)**.

2.2. Total count of different microorganisms. The enumeration of the microbial flora of each sample was carried out on nutrient agar (Atlas, 2006a), starch nitrate agar (Aghighi *et al.*, 2004) and Czapek's agar (Atlas, 2006b) media to count bacteria, actinobacteria and fungi, respectively. Aliquots (0.1 ml) from dilutions 10^{-1} to 10^{-5} were spread onto plates and liquefied medium was poured over the inoculum. After incubation at 37°C for 24 h (for bacteria) and 5-7 days (for actinomycetes and fungi), the obtained colonies were counted in the plates that contained 30-300 colonies and the average was measured (Obuekwe & Semple, 2011).

2.3. Isolation of actinobacteria and fungi. Colonies of actinobacteria and fungi that showed different characteristics were picked up randomly using sterile inoculation loop and sub-cultured by quadruplicate streaking on the same medium on Starch Nitrate agar and Czapek's agar media respectively then incubated. Purification was done, when necessary, until pure cultures were obtained.

2.4. Preliminary identification of the tested microorganisms.

2.4.1. Actinobacterial isolates. The studied actinobacteria were identified tentatively on the basis of their colonial and cell morphology, production of pigments on different media, ability to grow on Czapek's agar, utilization of different carbon sources, tolerance to different concentrations of sodium chloride, sensitivity to streptomycin, growth at different temperatures and antimicrobial activity up to genus level according to Barka *et al.* (2016) using the keys described in Bergey's Manual of Systematic Bacteriology and the International Streptomyces Project (I.S.P) (Shirling & Gottlieb, 1966; Madigan *et al.*, 1997; Xu *et al.*, 2007; Whitman, 2012).

2.4.2. Fungal isolates. The studied fungi were identified based on colony morphology on Czapek's agar and potato dextrose agar (PDA) media as well as their microscopic characteristics, mycelium septation, types of spores and dimensions of their different structures, and growth at different temperatures according to Klich (2002), Watanabe (2002), and Humber (1997) up to species level. Dimensions of the different fungal structures were measured using J. D. Möller 2 mm stage micrometer.

3. Results and Discussion

Sinai desert is of great economic importance as a promising area in terms of mining because of its unique geochemistry and various rock types. Study the microbial diversity in such location is of great importance to examine the microbial flora inhabiting such an ancient and unique environment, their interaction with the environment and their unexplored potential for application in biotechnological processes.

3.1. Rock samples. The studied rock samples are of sedimentary type and belong to Um Bogma Formation (early Carboniferous age, 320 million years ago) . Wadi Naseib samples are calcareous dark shale with higher organic matter content, while Um Hamd sample is brownish ferruginous shale with carbonaceous organic material.

3.2. Enumeration of microorganisms in the tested rock sample. The heterotrophic plate count of the microorganisms present in the studied samples when cultured at 37 and 55 °C is shown in table 1. The total count of microorganisms was low which might be due to the extreme temperature shifts in the studied locations throughout the year, the high concentration of heavy metal and radionuclides, low humidity and the low organic matter content (**El Galy *et al.*, 2008**). These factors does not favor microbial growth and should cause reduction in microbial count. The number of fungi was higher than that of bacteria except for sample III collected from Um Hamd mountain where bacterial count was higher when incubated at 55°C. Actinobacteria and fungi could not be isolated from the tested samples at a temperature of 55°C on starch nitrate agar and Czapek’s agar respectively. Our results are in accordance with **Brewer and Fierer (2018)** who stated that exposed rock surfaces present unique challenges for microbial growth and survival due to being subjected to high levels of UV radiation, frequent desiccation and limited resource availability. They also indicated that several factors can affect the rock-associated microbial communities, including climate, rock type, geological location and age of rock surface.

Table 1: Total count (CFU/g) of the microbial populations present in the tested samples.

Sample	I		II		III	
	37	55	37	55	37	55
Bacteria	60±20	20±2	400±10	0	80±20	300±10
Actinobacteria	ND*	0	40±10	0	5	0

Fungi	100	0	1800±40	0	80±20	0
-------	-----	---	---------	---	-------	---

*ND: not determined

3.3. Isolation of different actinobacteria and fungi. Our study enabled the isolation of 10 actinobacteria and 10 fungi. They were assigned the following symbols: A for actinobacteria, F for fungi, I, II and III according to the sample from which they were obtained and Arabic numerical to differentiate isolates obtained from the same sample.

3.4. Identification of the tested microorganisms.

3.4.1. Actinobacterial isolates. The studied actinobacterial isolates were aerobic, Gram-stain-positive, non-acid–alcohol-fast actinobacteria that have chalky appearance and form an extensively branched substrate mycelium which rarely fragments. The aerial mycelium forms long chains of spores (>15). They produced a wide variety of pigments responsible for the color of the vegetative (grey, white, beige, colorless, pink and salmon red) and aerial mycelia (white and grey). Colored diffusible pigments were also formed by some isolates (AII-1, AII-2, AII-3, AII-4, AII-5 and AIII-1) on ISP7. Spore chain was either rectus flexibilis, spiral, mono or verticillate. Phenotypic characteristics showed that all the tested isolates belong to genus *Streptomyces* according to **Whitman (2012)**. The cultures on starch nitrate agar plates and the pictures of the coverslip cultures stained using Gram staining are shown in Figure 2 and their description is illustrated in tables 2 and 3.

All the isolates were capable of growth on Czapek’s agar. They were also halotolerant as they were capable of resisting sodium chloride (2.5-7%). All were sensitive to 50 µg ml⁻¹ streptomycin when tested on Bennet’s agar. None were capable of growth at 55°C (except for isolate AII-2, AII-6 and AII-7 that gave weak growth). All were capable of growth at 25°C except isolate AIII-2 (isolate AI-1 and AII-5 gave weak growth). Only two isolates didn’t show any antimicrobial activity when tested on nutrient agar (AII-2, AII-6 and AIII-2), while all the rest showed either antibacterial activity (AII-1, AII-4 and AII-7) or antifungal activity (AII-3) as well as the dual activities (AI-1, AII-5 and AIII-1) with different efficacies. None of the tested isolates was capable of using salicin as a carbon source. The tested actinobacteria had different preference to the tested carbon sources. The biochemical characteristics are shown in tables 4 and 5.

There are former studies that indicated presence of bacteria belonging to the genus *Streptomyces* on rock surfaces and other extreme environments. These studies include the work of **Mohamed et al. (2001)** isolated five isolates belonging to the genus *Streptomyces* (*S. taurus* Si-4, *S. lateritius* Si-6, *S. mauvecolor* Si-9, *S. melanogenes* Si-11 and *Streptomyces* sp. Si-1) from Sinai sandy soil that showed the ability to tolerate sodium chloride concentrations of 9-12 %. Also, **Valan Arasu et al. (2009)** who isolated *Streptomyces* sp. strain ERI-3 from a forest rock soil sample collected from the Western Ghats, Tamil Nadu, India. Moreover, **Hamdali et al. (2010)** who isolated *Streptomyces lividans* and *S. griseus* from Moroccan phosphate mines with the ability to use the insoluble ground hydroxyapatite and produce bio-phosphate which suggests their potential to develop bio-phosphate fertilizer. Also, **Hewedy et al. (2013)** was capable of isolating 10 isolates of actinobacteria belonging to the genus *Streptomyces* from soil samples collected from Abu Thor, Sinai, Egypt that showed variable efficiencies in the bioleaching of both Rare Earth Element and uranium. Finally, **Abou-Dobara et al. (2017)** isolated *Streptomyces violaceoruber* ES from a soil sample collected from Sharm El-Sheikh, Sinai, Egypt which showed antibacterial activity.

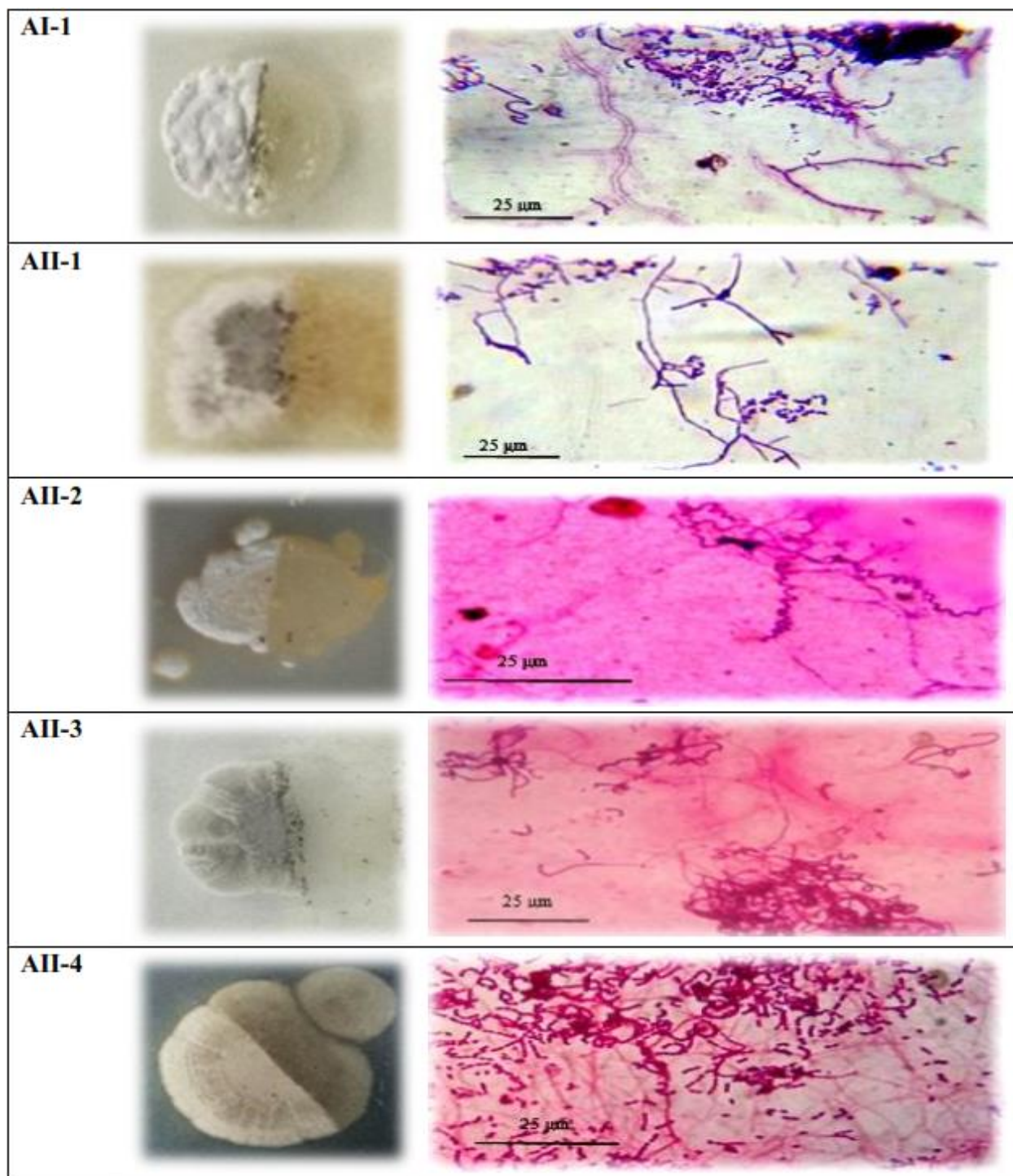


Figure 2: Colony morphology and pictures of Gram-stained coverslips of the studied actinobacteria.

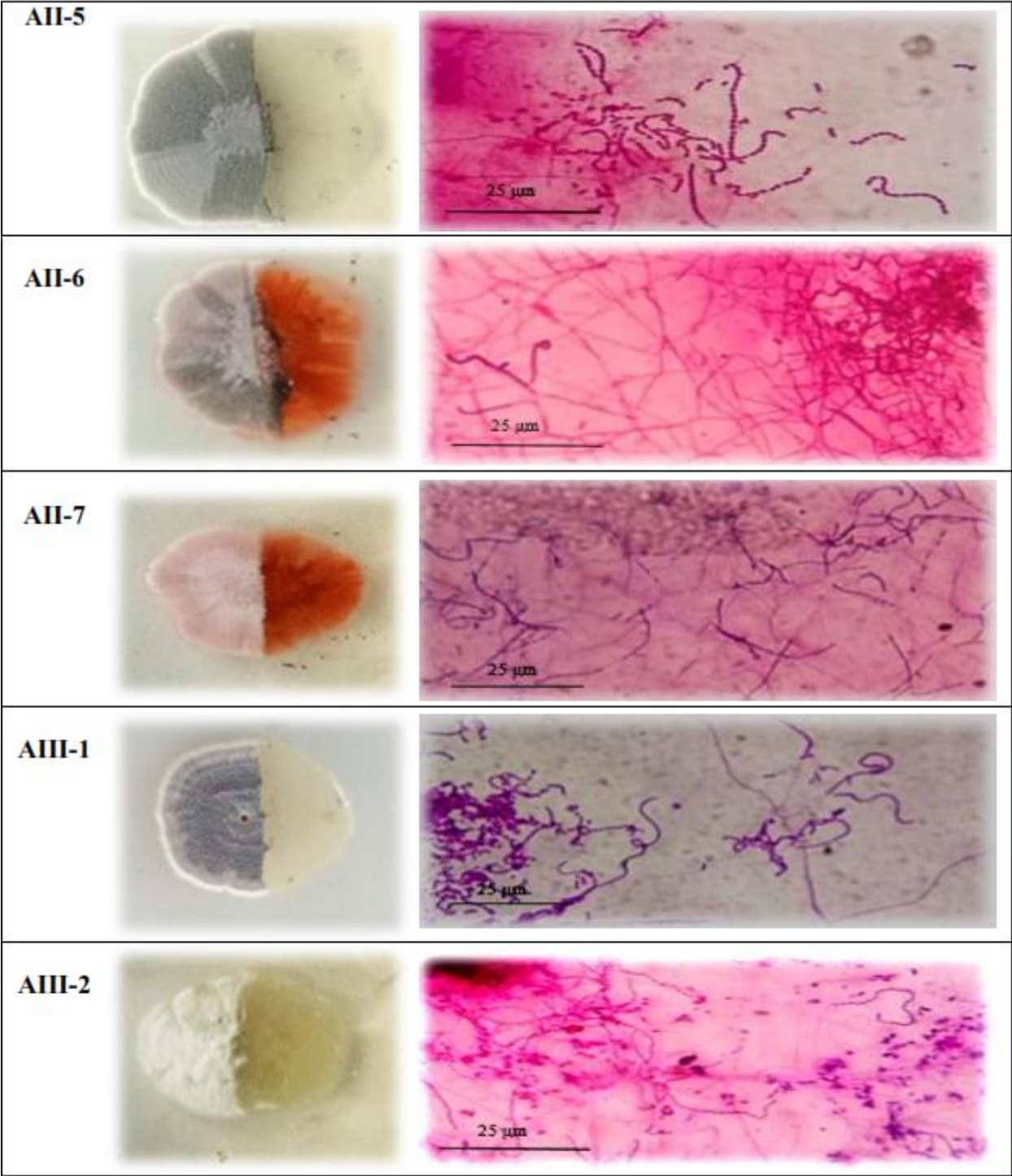


Figure 2: Continued.

Table 2: Cultural characteristics of the studied actinobacteria.

Isolate	Culture media												
	Starch nitrate agar				ISP6				ISP7				Czapek's agar
	Aerial mycelium	Substrate mycelium	Diffusible pigment	Appearance	Aerial mycelium	Substrate mycelium	Diffusible pigment	Appearance	Aerial mycelium	Substrate mycelium	Diffusible pigment	Appearance	
AI-1	Pale Grey	Pale beige	-	C	White	Pale beige	-	V	White	Pale beige	-	V	+
AII-1	Grey with white edge	Beige	-	V	White	Beige	-	C	Grey	Beige	Brown	V	+
AII-2	Greyish white	Pale beige	-	C	White	Beige	-	C	Grey	Colorless	Brown	V, F	+
AII-3	Grey	Colorless	-	V	Grey	Beige	-	V	Grey	Beige	Brown	V	+
AII-4	Pale grey	Beige	-	V	Grey	Beige	-	C	Grey	Beige	Brown	V	+
AII-5	Grey	Colorless	-	V	Pale grey	Beige	-	C	Grey	Beige	Brown	V	+
AII-6	Grey	Salmon red	-	V	Grey	Brown	-	V	Grey	Dark pink	-	V	+
AII-7	White	Salmon red	-	V	White	Beige	-	C	White	Dark pink	-	V	+
AIII-1	Grey	Greenish white	-	V	Grey	Pale brown	-	V	Grey	Beige	Brown	V	+
AIII-2	White	Beige	-	C	White	Pale beige	-	V	White	Pale beige	-	V	+

Symbols: C, Cottony; V, Velvety; +, growth; -, no growth; F, fairy rings.

Table 3: Description of spore chains of the studied actinobacteria.

Isolate	Spore chain length	Spore chain morphology	Spore shape
AI-1	Long	RF	Rod
AII-1	Long	RF	Rod
AII-2	Long	Mono-VE – S	Rod
AII-3	Long	Mono-VE – RF	Rod
AII-4	Long	Bi-VE - RF	Spherical
AII-5	Long	RF	Rod
AII-6	Long	Mono-VE - S	Rod
AII-7	Long	Mono-VE - S	Rod
AIII-1	Long	Bi-VE - RF	Rod
AIII-2	Long	RF	Rod

Symbols: Spore chain morphology indicated as RF (Rectus Flexibilis), S (Spiral) and VE (Verticil).

Table 4: Biochemical tests of the studied actinobacteria.

Characteristic	Isolate										
	AI-1	AI-1	AI-2	AI-3	AI-4	AI-5	AI-6	AI-7	AII-1	AII-2	
Sodium chloride tolerance:											
NaCl concentration (%)	2.5	+	+	+	+	+	+	+	+	+	+
	5	W	+	+	+	W	+	+	+	+	+
	7.5	-	-	±	±	-	W	-	±	±	±
	10	-	-	-	-	-	-	-	-	-	-
Sensitivity to streptomycin (50 µg ml ⁻¹)	S	S	R	S	S	S	S	S	S	S	
Growth at 55°C	-	-	W	-	-	-	W	W	-	-	
Growth at 25°C	W	+	+	+	+	W	+	+	+	-	
Antimicrobial activity against:											
1. <i>Bacillus subtilis</i>	+	+	-	-	+	-	-	+	+	-	
2. <i>Pseudomonas aeruginosa</i> ATCC 9027	+	+	-	-	+	+	-	+	+	-	
3. <i>Staphylococcus aureus</i> ATCC 6538	+	+	-	-	-	-	-	+	+	-	
4. <i>Escherichia coli</i> ATCC 8739	-	+	-	-	-	-	-	-	-	-	
5. <i>Candida albicans</i>	+	-	-	+	-	+	-	-	+	-	
6. <i>Aspergillus flavus</i>	+	-	-	+	-	+	-	-	+	-	

Symbols: +, growth; -, no growth; ±, doubtful; w, weak growth; R, resistant; S, sensitive.

Table 5: Utilization of carbon source by the studied actinobacteria.

Carbon source	Isolate									
	AI-1	AII-1	AII-2	AII-3	AII-4	AII-5	AII-6	AII-7	AIII-1	AIII-2
D-Glucose (positive control)	+	+	+	+	+	+	+	+	+	+
Dextrose	-	+	W	+	+	+	+	+	+	-
D-Fructose	-	+	W	+	+	+	+	+	+	-
Galactose	-	+	+	+	+	+	+	+	+	-
D-Mannitol	-	+	W	+	+	+	+	+	+	-
Inositol	-	+	W	+	+	+	+	+	+	-
L-Arabinose	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mannose	-	+	W	+	+	+	+	+	+	±
Salicin	-	-	-	-	-	-	-	-	-	-
D-Xylose	-	+	+	+	+	+	+	+	+	-
Lactose	-	+	+	+	+	+	+	+	+	W
Sucrose	W	+	W	+	+	+	+	+	+	W
Maltose	-	-	+	-	-	+	+	+	+	-
Raffinose	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Starch	+	+	+	+	+	+	+	+	+	+

Symbols: +, growth; -, no growth; ±, doubtful; w, weak growth; ND, test not determined.

3.4.2. Fungal isolates. Examination of the studied isolates revealed that they have septate mycelium with a vesicle at the end of sporophore bearing catenate conidiospores, in addition to ascospores. Colony diameter ranged from 12 to > 50 mm after 10 days of incubation. Thus, they were all from the phylum Ascomycota and they belong to the genus *Aspergillus* according to **Samson et al. (2014)**. They were found to be belonging to 8 species (*A. terreus*, *A. flavus*, *A. candidus*, *A. medius*, *A. nidulans*, *A. niveus*, *A. tamaritii* and *A. fumigatus*) according to **Humber (1997)**, **Klich (2002)** and **Watanabe (2002)**. The pictures of the coverslips of the studied fungi under the light microscope as well as cultures on Czapek's agar and PDA are shown in Fig. 4-12. Cultural and microscopic characteristics are shown in tables 6-8.

All the studied fungi were capable of growth at 25°C except isolate FII-3. FIII-2 was capable of growth at 55°C, while FII-2, FII-3 and FII-6 couldn't grow and the rest gave only weak growth. The fungal isolates had either subclavate or globose, biseriate or uniseriate vesicle and the conidial head was either radiate or columnar. They formed globose conidia with different colors and globose, ovoid to ellipsoidal ascospores. Hülle cells was observed in case of isolates FII-3 and FII-6, aleuriospores were observed in case of isolates FI-1, FI-2, FII-1 and FII-4, while arthrospores were formed only by isolate FIII-1.

Our results are in accordance with **Gonçalves et al. (2016)** who studied the diversity of rock-associated fungi from Atacama Desert and obtained 81 fungal isolates that belonged to the Ascomycota taxa as proved by sequencing different regions of DNA. They found that most of the isolated fungi belonged to the genera *Cladosporium*, *Penicillium*, *Aspergillus* and *Fusarium*. Other studies also involving rock colonizing fungi indicates the presence of fungi that belong to the phylum Ascomycota in the studied samples (**Li et al., 2016; Brewer & Fierer, 2018**). Moreover, several authors proved that different species belonging to the genus *Aspergillus* were capable of adapting desert environments and also applied in the biomining of several metals (**Santhiya and Ting, 2006; Siddiqui et al., 2009; Thosar et al., 2014**).

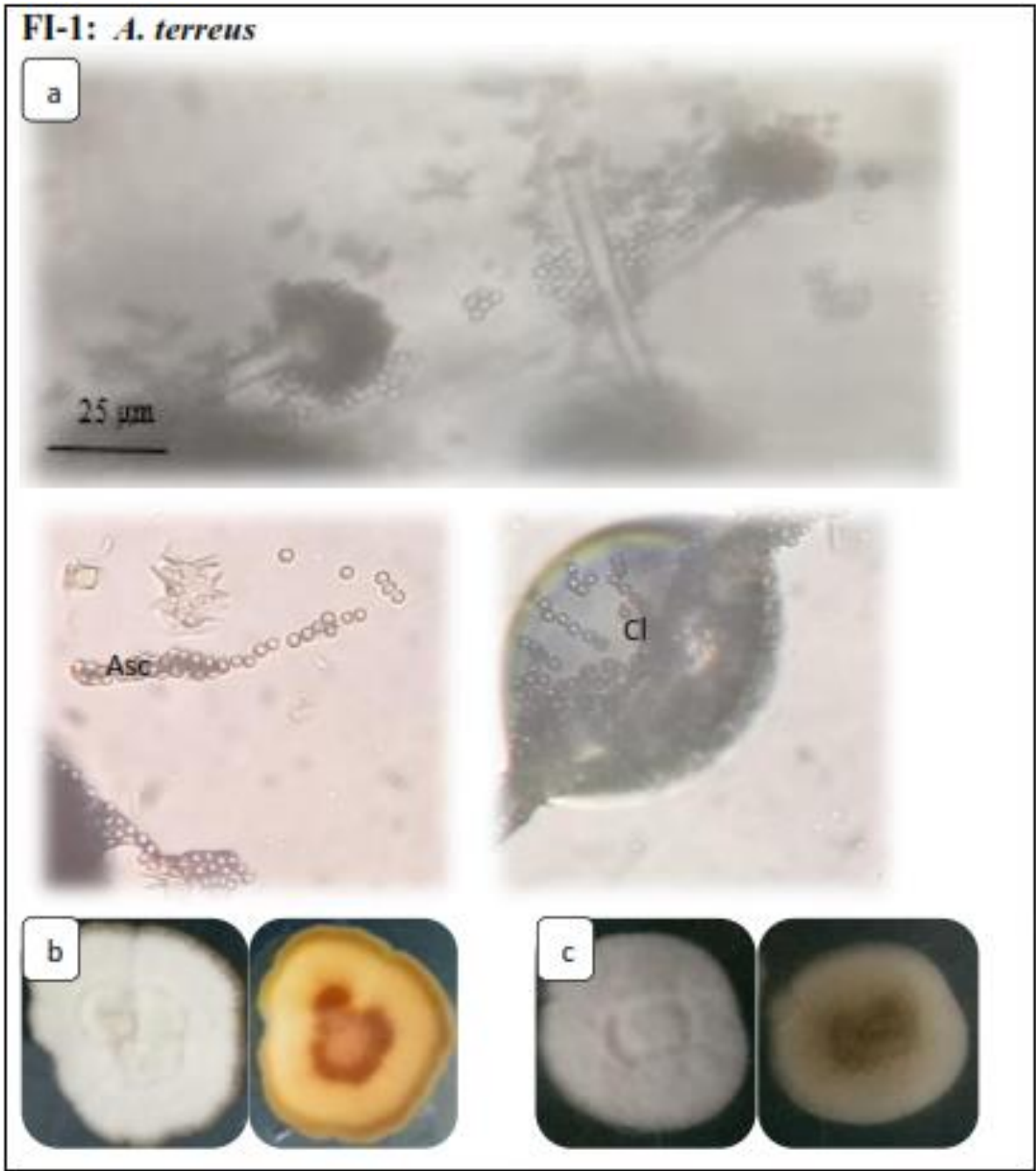


Figure 3: Microscopic and culture pictures of the fungal isolate FI-1. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; Cl, cleistothecium; Asc, ascospore.

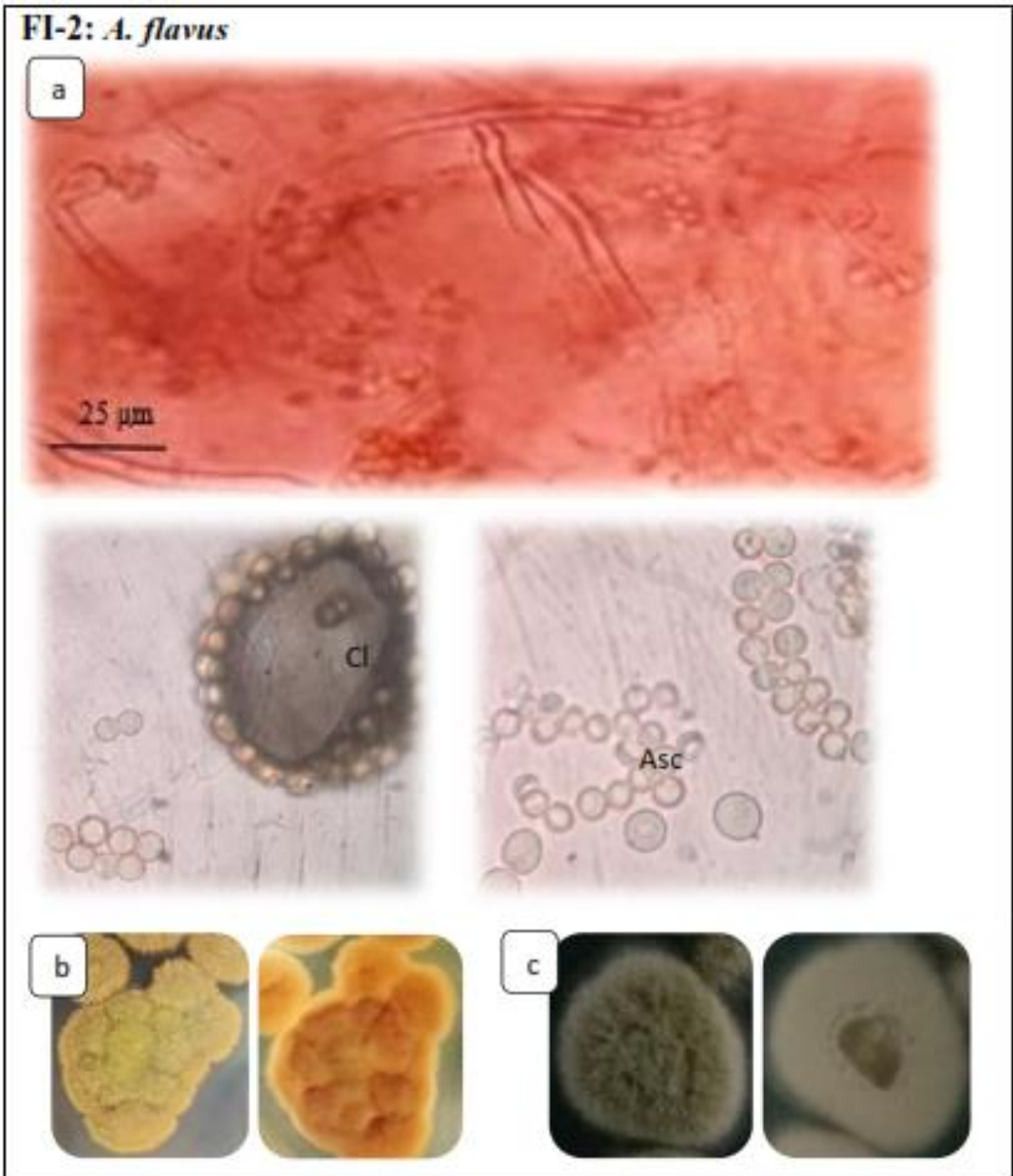


Figure 4: Microscopic and culture pictures of the fungal isolate FI-2. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; Cl, cleistothecium; Asc, ascospore.

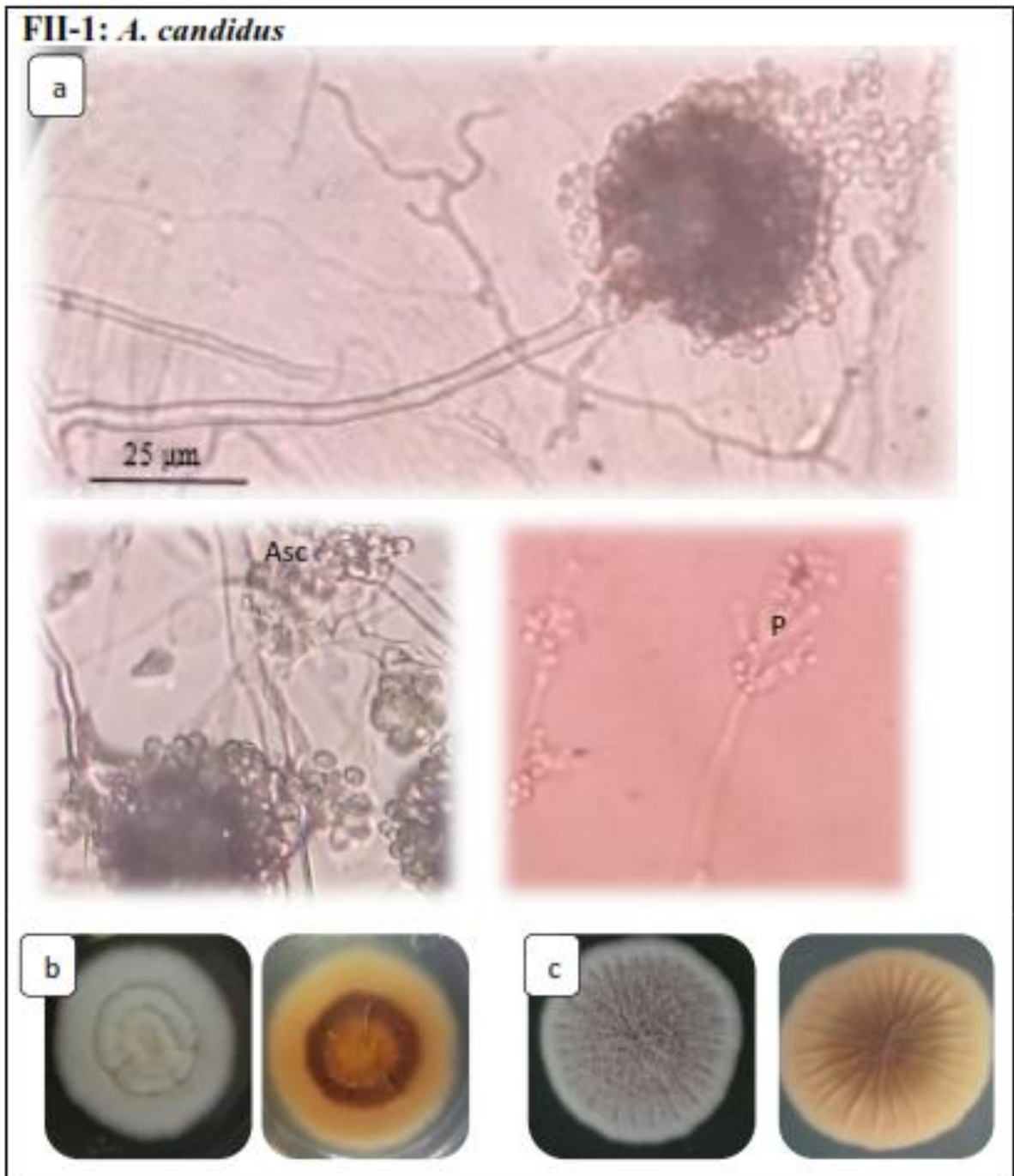


Figure 5: Microscopic and culture pictures of the fungal isolate FII-1. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; Asc, ascospore; P, reduced *Penicillium*-like structures.

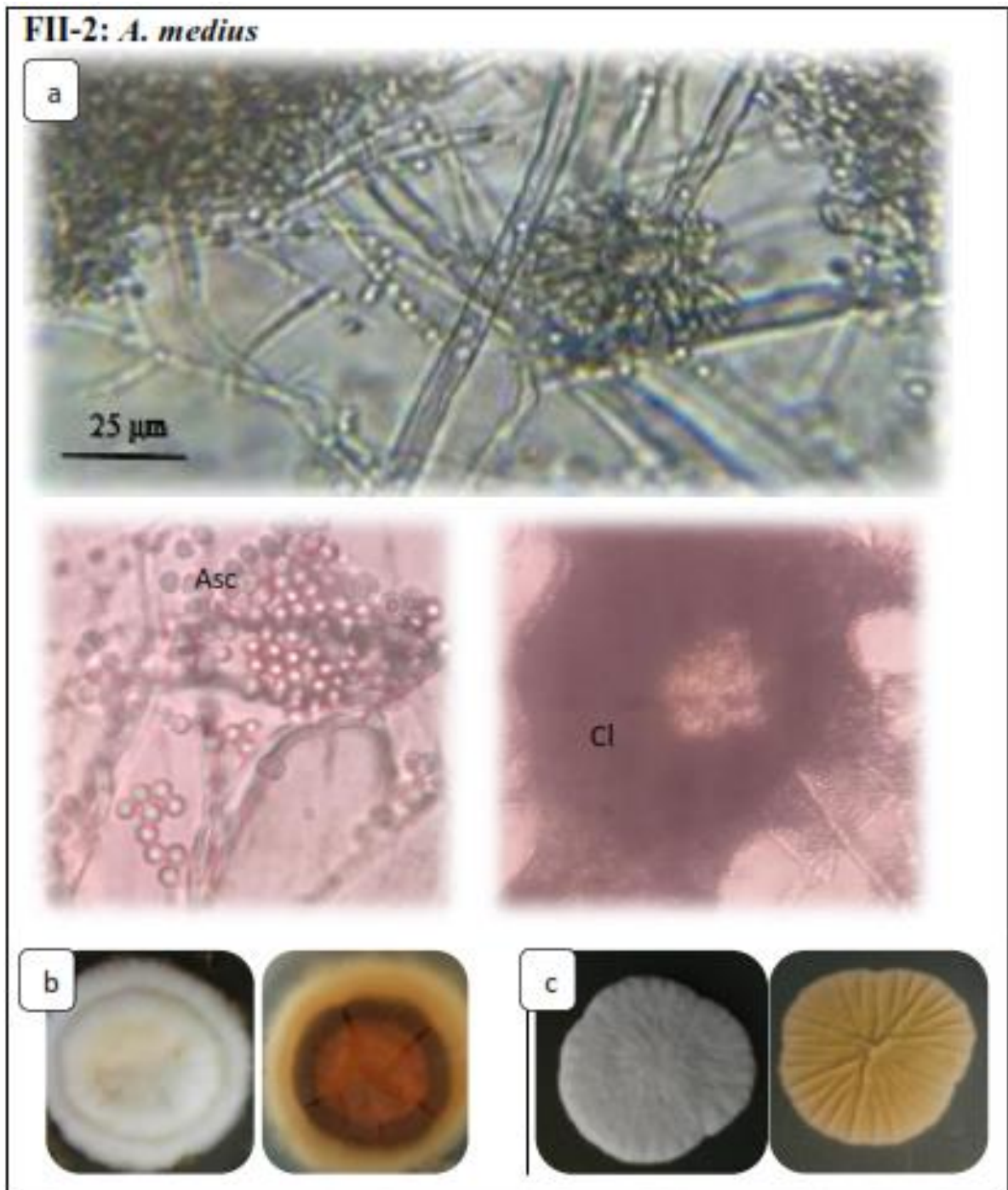


Figure 6: Microscopic and culture pictures of the fungal isolate FII-2. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; Asc, ascospore; Cl, cleistothecium.

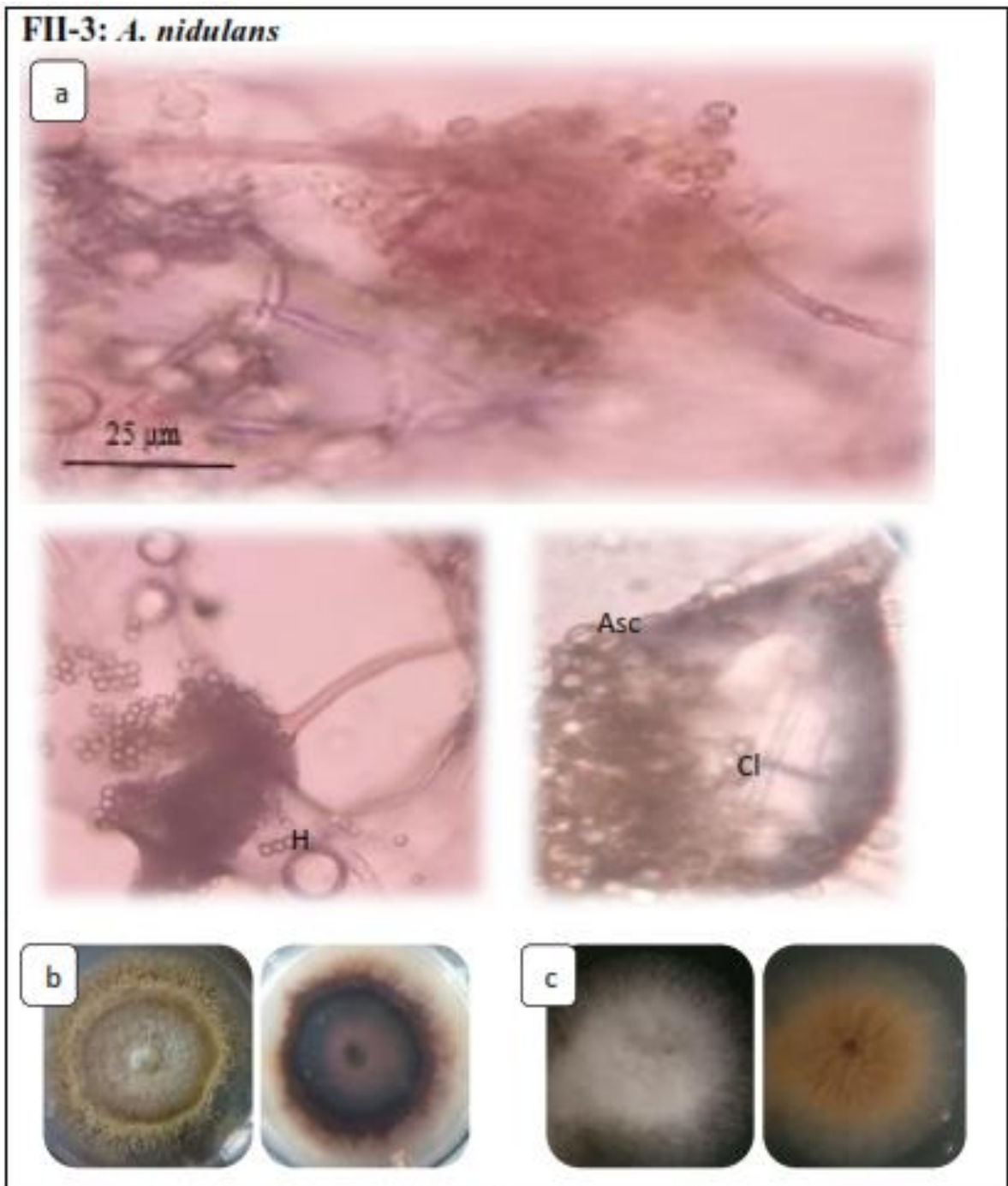


Figure 7: Microscopic and culture pictures of the fungal isolate FII-3. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; H, hülle cells; Cl, cleistothecium; Asc, ascospores; Al, aleuriospore; G, germinating conidiophore.

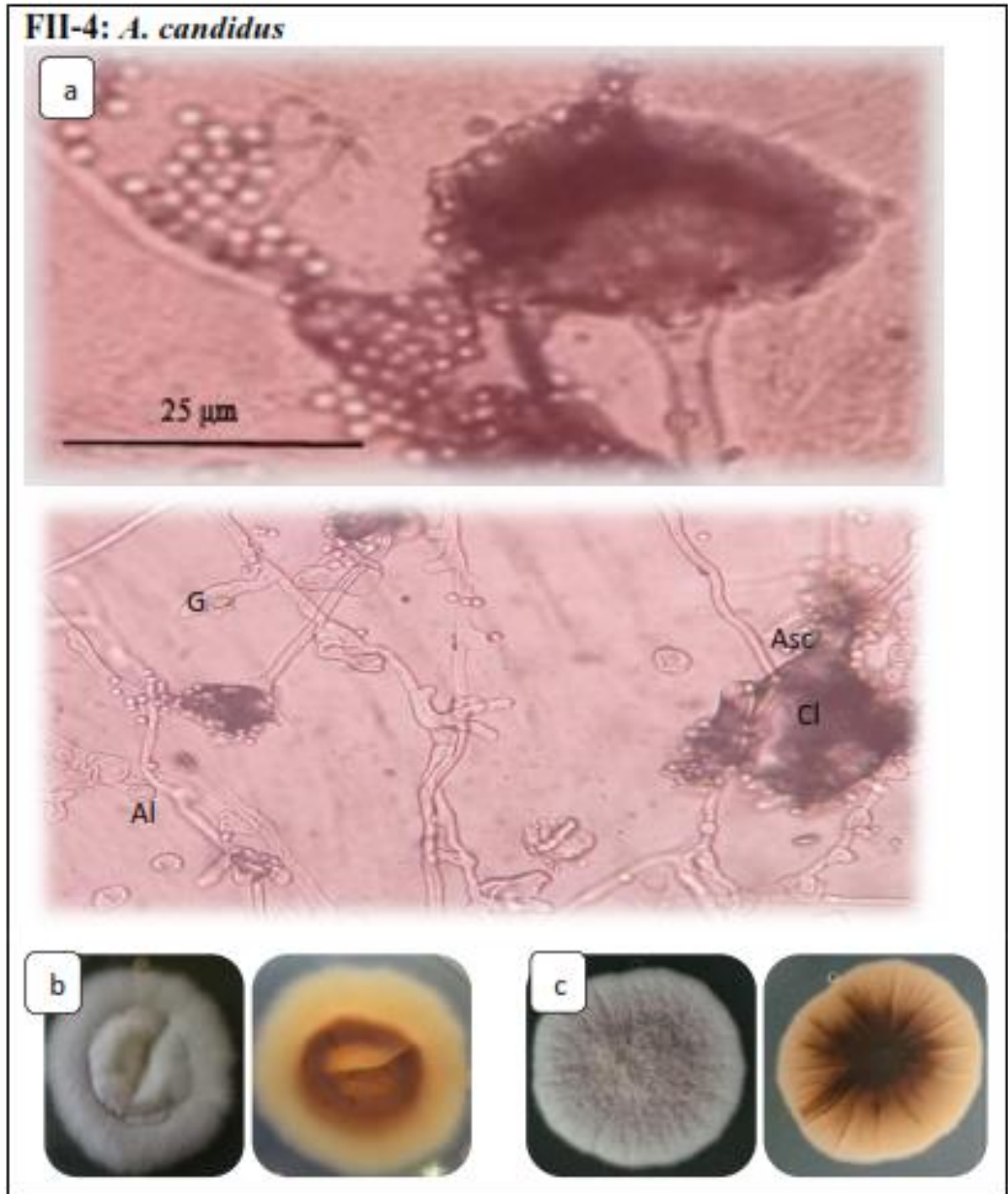


Figure 8: Microscopic and culture pictures of the fungal isolate FII-4. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; Cl, cleistothecium; Asc, ascospores; Al, aleuriospore; G, germinating conidiophore.

FII-5: *A. niveus*

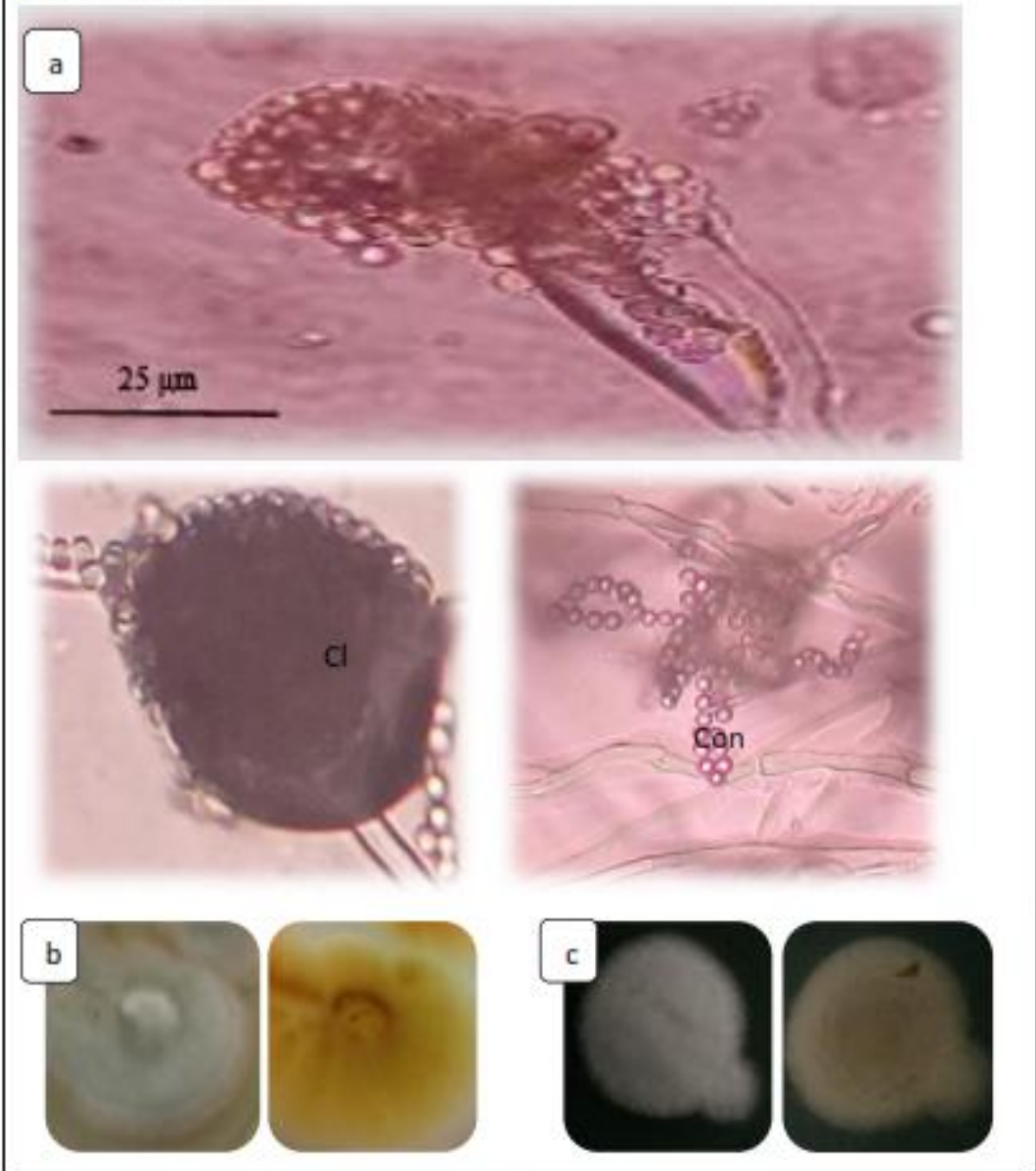


Figure 9: Microscopic and culture pictures of the fungal isolate FII-5. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; Con, conidiospore; Cl, cleistothecium.

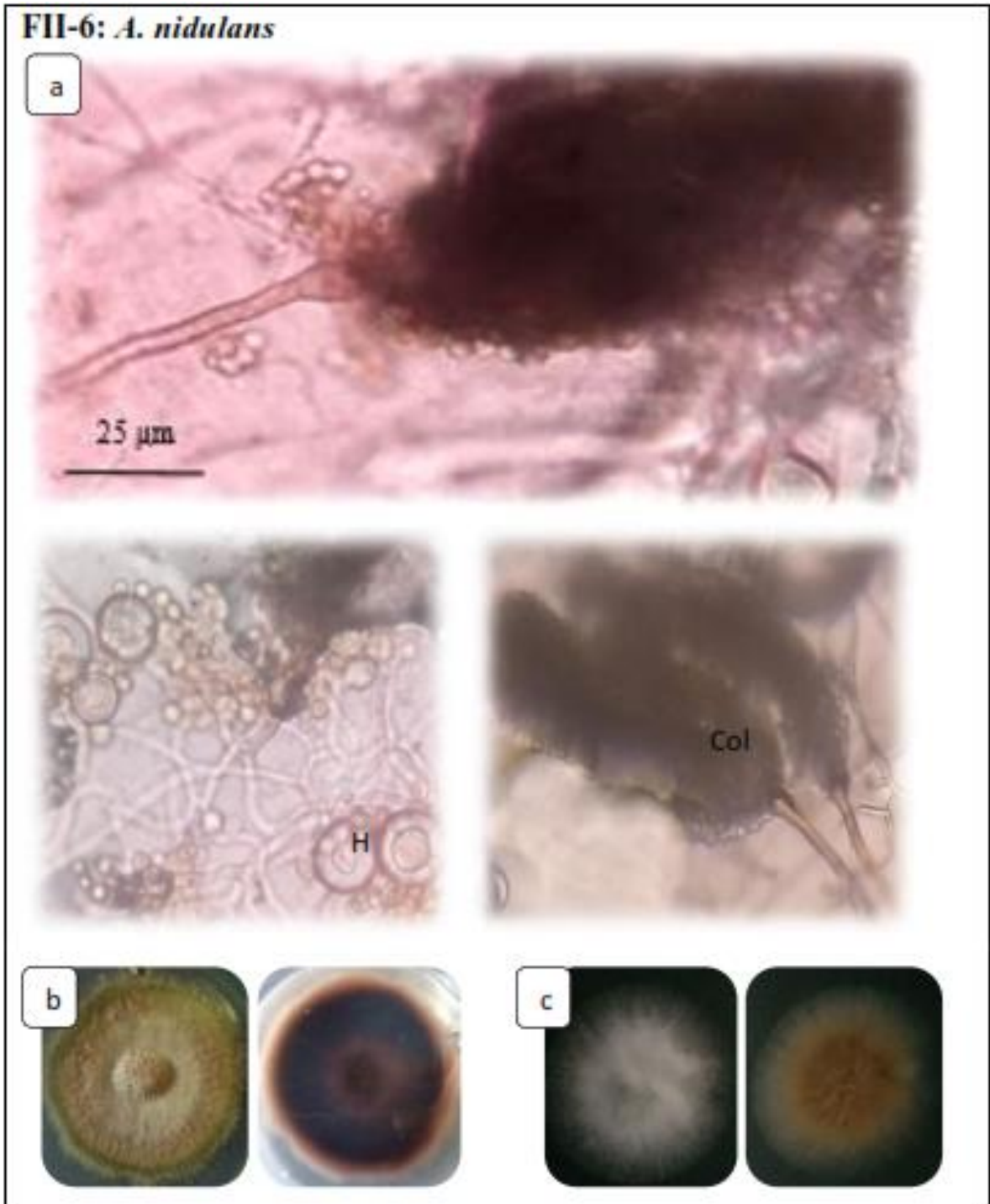


Figure 10: Microscopic and culture pictures of the fungal isolate FII-6. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; H, hülle cells; Col, columnar head.

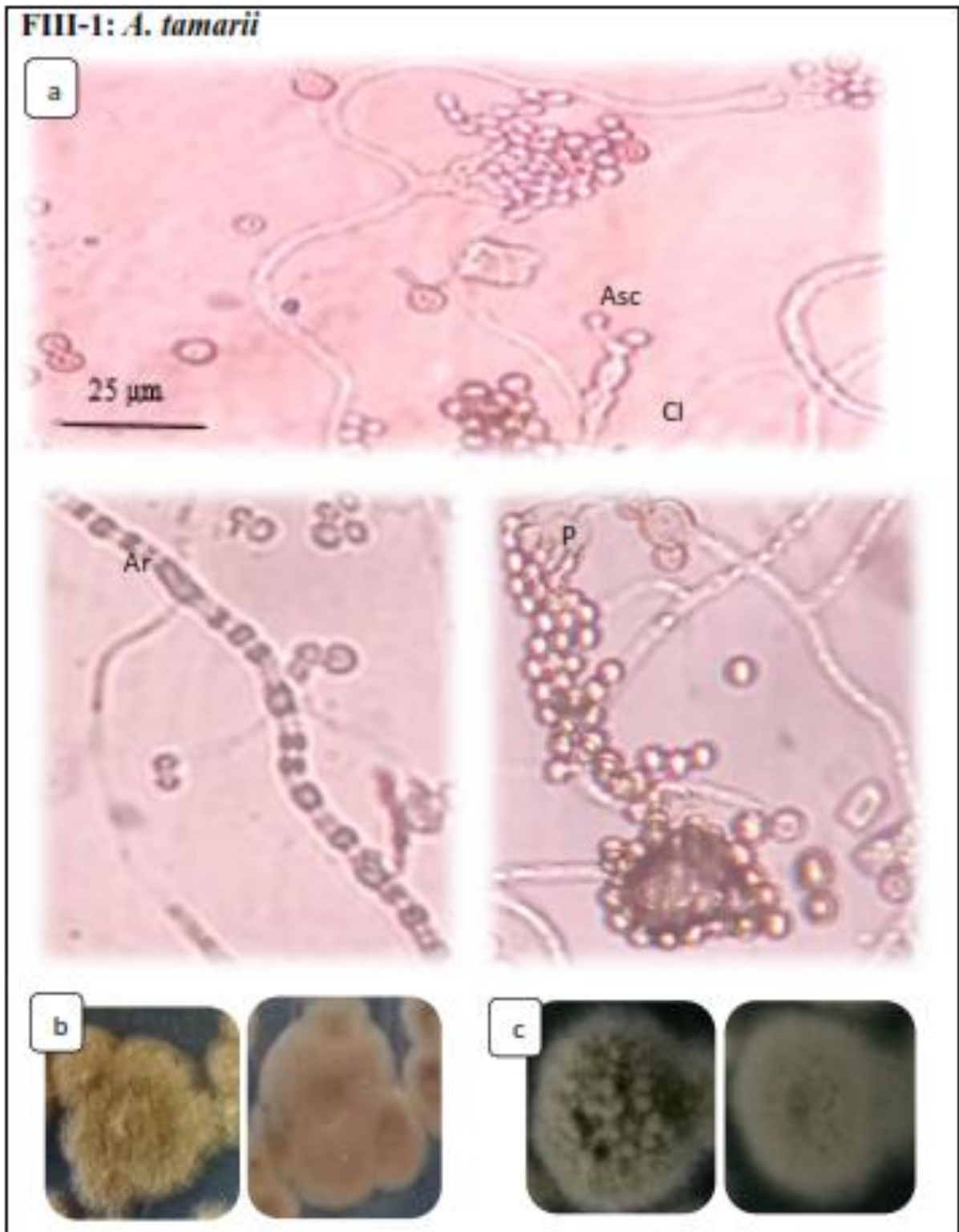


Figure 11: Microscopic and culture pictures of the fungal isolate FIII-1. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; Ar, arthrospores; P, phialide; Asc, ascospores; Cl, cleistothecium.

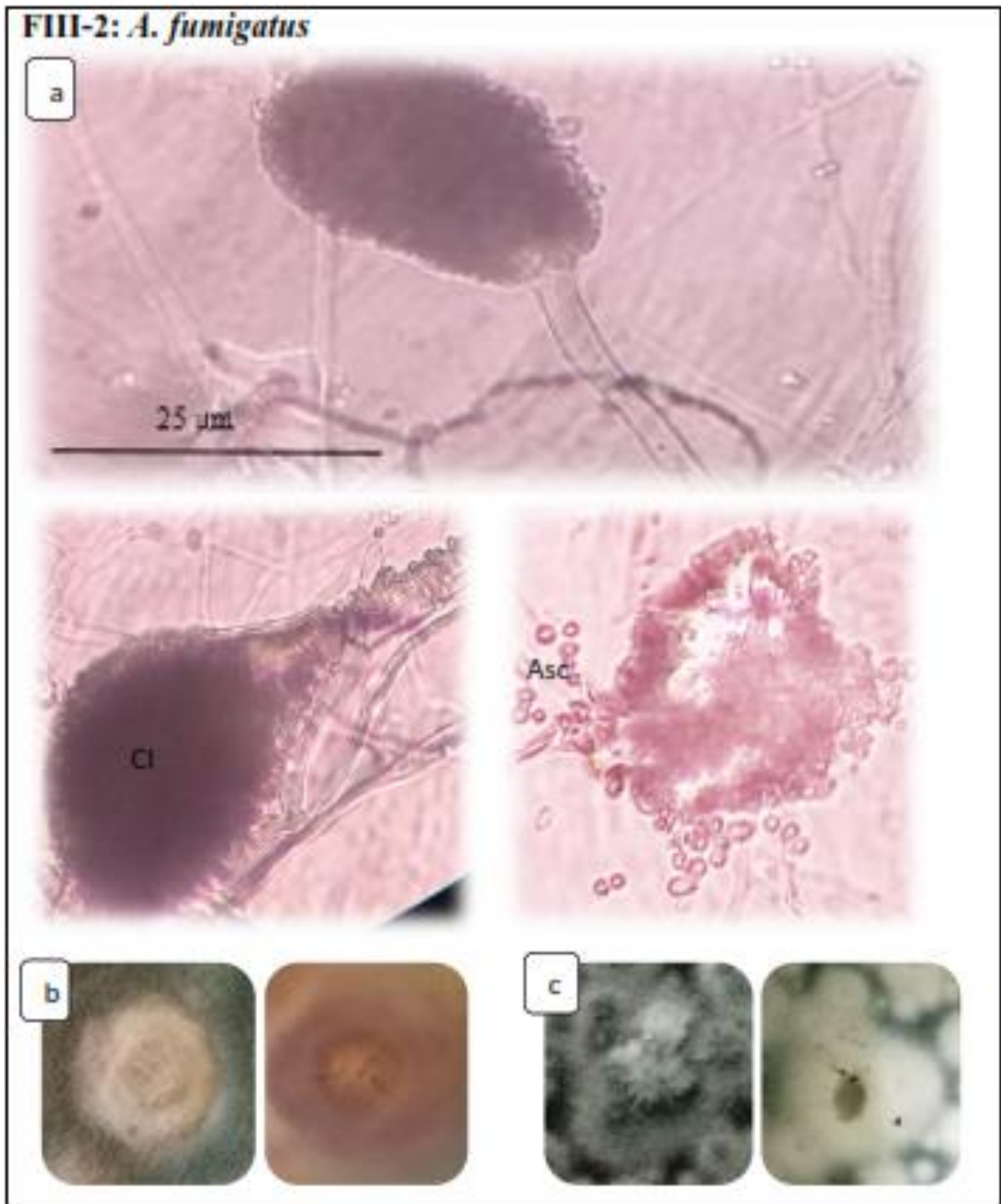


Figure 12: Microscopic and culture pictures of the fungal isolate FIII-2. a, microscopic picture; b, colony forward and reverse on Czapek's agar; c, colony forward and reverse on PDA; Asc, ascospores; Cl, cleistothecium.

Table 6: Cultural characteristics of the tested fungi.

Isolate	Cultural features on Czapek's agar (after 10 days of incubation)			Other features				Name
	Colony diameter (mm)	Colony surface	Colony reverse	Growth rate	Spore detachment	Growth at 25°C	Growth at 55°C	
FI-1	23.8±4.8	White wrinkled colonies – cinnamon-buff or purplish sclerotia with yellowish exudates – azonate at first then zonate at margin.	Alternating yellow and brown concentric ring pattern.	Slow	Hard	+	±	<i>A. terreus</i>
FI-2	22.6±1.2	Velvety, dark yellowish green colonies with white margin.	Pale yellowish brown.	Medium	Easy	+	±	<i>A. flavus</i>
FII-1	30.9±2.4	Yellowish white colonies.	Star-shaped striations showing with alternating yellow and olive to brownish orange concentric ring pattern.	Slow	Hard	+	±	<i>A. candidus</i>
FII-2	28.6±2.4	White colonies with yellow edges which turns green with old - yellow exudates.	Star-shaped striations showing with alternating yellow and olive to brownish green concentric ring pattern.	Slow	Hard	+	-	<i>A. medius</i>
FII-3	42.3±1.9	Brownish green colonies, brown exudates.	Dark reddish brown to purple with concentric ring pattern.	Fast	Hard	-	-	<i>A. nidulans</i>

Table 6: Continued.

Isolate	Cultural features on Czapek's agar (after 10 days of incubation)			Other features				Name
	Colony diameter (mm)	Colony surface	Colony reverse	Growth rate	Spore detachment	Growth at 25°C	Growth at 55°C	
FII-4	35.7±2.4	White colonies – colorless exudates.	Star-shaped striations showing with alternating yellow and olive to brownish orange concentric ring pattern.	Slow	Hard	+	±	<i>A. candidus</i>
FII-5	31.8±6.8	Dull orange-white colonies.	Slight brownish.	Slow	Hard	+	±	<i>A. niveus</i>
FII-6	38.5±3.9	Brownish green colonies with colorless exudates.	Dark reddish brown to purple with concentric ring pattern.	Fast	Hard	+	-	<i>A. nidulans</i>
FIII-1	12.9±1.5	Flat, dark olive green colonies, wrinkled mycelial growth.	Pale brown.	Fast	Easy	+	±	<i>A. Tamaritii</i>
FIII-2	Whole plate	Bluish green colonies, white mycelia.	Pale reddish brown.	Fast	Easy	+	+	<i>A. fumigatus</i>

Table 7: Microscopic features of the tested fungi.

Isolate	<i>Hypha</i>	<i>Conidiophore</i>	<i>Vesicle</i>	<i>Conidial head</i>	<i>Vesicle Serration</i>	<i>Conidia</i>	<i>Ascospores</i>	<i>Hülle cells</i>	<i>Aleurioconidia</i>	<i>Arthrospores</i>
FI-1	Septate	Colorless short smooth-walled	Subclavate	Radiate	Biseriate	Globose	Globose ascospores having longitudinal crests	-	+	-
FI-2	Septate	Erect, simple, rough in the surface	Globose	Columnar	Biseriate	Pale green	Globose ascospores having longitudinal crests	-	+	-
FII-1	Septate	Hyaline	Globose to subclavate	Radiate	Biseriate	Globose, thick-walled	Globose ascospores having longitudinal crests	-	+	-
FII-2	Septate	Tall, thin-walled, smooth, with one to three branches	Subclavate	Radiate	Uniseriate	Globose, thick-walled	Globose ascospores having longitudinal crests	-	-	-
FII-3	Septate	Geniculate, short	Globose to subclavate	Radiate to loosely columnar	Biseriate	Globose rough-walled conidia	Globose ascospores having longitudinal crests	+	-	-

Table 7: Continued.

Isolate	<i>Hypha</i>	<i>Conidiophore</i>	<i>Vesicle</i>	<i>Conidial head</i>	<i>Vesicle Serration</i>	<i>Conidia</i>	<i>Ascospores</i>	<i>Hülle cells</i>	<i>Aleurioconidia</i>	<i>Arthrospores</i>
FII-4	Septate	Hyaline	Globose to subclavate	Radiate	Biseriate	Globose, thick-walled	Globose ascospores having longitudinal crests	-	+	-
FII-5	Septate	Hyaline, long	Subclavate	Radiate	Biseriate	Globose	Globose ascospores having longitudinal crests	-	-	-
FII-6	Septate	Geniculate, short	Globose to subclavate	Columnar	Biseriate	Globose rough-walled conidia	Globose ascospores having longitudinal crests	+	-	-
FIII-1	Septate	Pale brown, rough walled.	Globose to subclavate	Columnar	Uniseriate	Globose or subglobose, smooth, double-walled	Ovoid to ellipsoidal ascospores having longitudinal crests	-	-	+
FIII-2	Septate	Hyaline	Globose to subclavate	Columnar	Uniseriate	Globose, slightly echinulate	Globose ascospores having longitudinal crests	-	-	-

Table 8: Dimensions of structures of the tested fungi (μm).

Isolate	Name	Conidiophore	Vesicle	Phialides	Conidia
FI-1	<i>A. terreus</i>	320 \pm 0.0	4.25 \pm 0.4	4.75 \pm 0.4	2.5 \pm 0.0
FI-2	<i>A. flavus</i>	250 \pm 0.0	13.75 \pm 3.2	9 \pm 2.8	5.5 \pm 0.7
FII-1	<i>A. candidus</i>	275 \pm 35.4	7 \pm 0.7	8 \pm 0.0	3.5 \pm 0.7
FII-2	<i>A. medius</i>	300 \pm 70.7	13.5 \pm 2.1	9 \pm 1.4	6 \pm 0.0
FII-3	<i>A. nidulans</i>	85 \pm 7.1	9.75 \pm 0.4	8 \pm 0.0	3.5 \pm 0.7
FII-4	<i>A. candidus</i>	170 \pm 14.1	7 \pm 0.7	8.25 \pm 0.4	2.75 \pm 0.4
FII-5	<i>A. niveus</i>	280 \pm 56.6	9.75 \pm 0.4	6.5 \pm 0.7	2.25 \pm 0.4
FII-6	<i>A. nidulans</i>	32.5 \pm 10.6	8 \pm 0.7	6.5 \pm 0.7	3.75 \pm 0.4
FIII-1	<i>A. tamarii</i>	50 \pm 14.1	8.5 \pm 0.7	8 \pm 1.4	4 \pm 0.0
FIII-2	<i>A. fumigatus</i>	125 \pm 7.1	24.5 \pm 0.7	6 \pm 0.0	2.75 \pm 0.4

4. Conclusions

Our present study enabled the isolation of 10 isolates of actinobacteria and 10 isolates of fungi from rock samples collected from 3 different locations in Sinai. Morphological, cultural as well as biochemical characterization showed that all the studied actinobacteria belong to the genus *Streptomyces* and all the studied fungi belong to the genus *Aspergillus*. We are now working molecular identification and phylogenetic studies of the isolated actinobacteria and fungi to explain the phylogenetic relationships and the relatedness between the studied isolates in each group. We are also working examining their potential in uranium-biomining. Furthermore studies should be undertaken on a detailed basis to identify the antimicrobial compound from the isolated *Streptomyces* isolates and their efficacies against other pathogenic microorganisms could be useful for pharmaceutical applications.

5. References

Abou-Dobara, M. I., El-Sayed, A. & Omar, N. F. (2017). *Streptomyces violaceoruber* ES: A Producer of Bioprospective Metabolite for Rapid and Green Synthesis of Antibacterial Silver Nanoparticles. *Egyptian Journal of Botany*, 57 (7th International Conf.), 31-43. <https://doi.org/10.21608/ejbo.2017.1017.1089>

- Abu Bakr, M. A.**, El Mezayen, A. M., Sherif, H. M. Y., El Nahas, H. A. & Ali, H. H. (2016). Geology and Radioactivity of the Paleozoic Rocks of Wadi El-Sahu Area, Southwestern Sinai, Egypt. *International Journal of Innovative Science, Engineering & Technology*, 3(6): 492-517.
- Afandy, A. H. E.**, Shata, A. E. & Halim, K. A. E. (2010). Geochemistry and Radioactivity of Abu Thora Formation Southwest Sinai, Egypt. *Sci. J. Fac. Sci. Minufya Univ.*, 24: 1-40.
- Aghighi, S.**, Shahidi Bonjar, G. H. & Saadoun, I. (2004). First report of antifungal properties of a new strain of *Streptomyces plicatus* (strain 101) against four Iranian phytopathogenic isolates of *Verticillium dahlia*, A new horizon in biocontrol agents. *Biotech*, 3(1): 90-97. <https://doi.org/10.3923/biotech.2004.90.97>
- Atlas, R. M. (2006a)**. *Handbook of Microbiological Media*. LLC: CRC Press, Taylor & Francis Group (p. 256).
- Atlas, R. M. (2006b)**. *Handbook of microbiological media for the examination of food* (2nd ed.). New York: CRC Press, Taylor & Francis Group, LLC (pp. 111 & 283).
- Barka, E. A.**, Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y. & Wezeld, G. P. v. (2016). Taxonomy, Physiology, and Natural Products of Actinobacteria. *Microbiol Mol Biol Rev*, 80(1): 1-43. <https://doi.org/10.1128/MMBR.00019-15>
- Berdy, J. (2005)**. Bioactive microbial metabolites. *The Journal of antibiotics*, 58(1): 1-26. <https://doi.org/10.1038/ja.2005.1>
- Bhatnagar, A. & Bhatnagar, M. (2005)**. Microbial diversity in desert ecosystems. *Current Science*, 89(1): 91-100.
- Bhatti, A. A.**, Haq, S. & Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant health. *Microbial Pathogenesis*, 111: 458-467. <https://doi.org/10.1016/j.micpath.2017.09.036>
- Brewer, T. E. & Fierer, N. (2018)**. Tales from the tomb: the microbial ecology of exposed rock surfaces. *Environ Microbiol*, 20(3): 958-970. <https://doi.org/10.1111/1462-2920.14024>
- El-Bialy, M. Z. & Shata, A. E. (2018)**. Geochemistry, petrogenesis and radioactive mineralization of two coeval Neoproterozoic post-collisional calc-alkaline and alkaline granitoid suites from Sinai, Arabian Nubian Shield. *Geochemistry*, 78(1): 15-39. <https://doi.org/10.1016/j.chemer.2017.12.001>

- El Galy, M. M.,** El Mezayn, A. M., Said, A. F., El Mowafy, A. A. & Mohamed, M. S. (2008). Distribution and environmental impacts of some radionuclides in sedimentary rocks at Wadi Naseib area, southwest Sinai, Egypt. *J Environ Radioact*, 99(7): 1075-1082. <https://doi.org/10.1016/j.jenvrad.2007.12.012>
- Gadd, G. M. (2017).** Fungi, Rocks, and Minerals. *Elements*, 13(3): 171-176. <https://doi.org/10.2113/gselements.13.3.171>
- Ghorbani-Nasrabadi, R.,** Greiner, R., Alikhani, H. A., Hamed, J. & Yakhchali, B. (2013). Distribution of actinomycetes in different soil ecosystems and effect of media composition on extracellular phosphatase activity. *J. Soil Sci. Plant Nutr.*, 13(1): 223-236. <https://doi.org/10.4067/S0718-95162013005000020>
- Gonçalves, V. N.,** Cantrell, C. L., Wedge, D. E., Ferreira, M. C., Soares, M. A., Jacob, M. R., Oliveira, F. S., Galante, D., Rodrigues, F., Alves, T. M. A., Zani, C. L., Junior, P. A. S., Murta, S., Romanha, A. J., Barbosa, E. C., Kroon, E. G., Oliveira, J. G., Gomez-Silva, B., Galetovic, A., Rosa, C. A. & Rosa, L. H. (2016). Fungi associated with rocks of the Atacama Desert: taxonomy, distribution, diversity, ecology and bioprospection for bioactive compounds. *Environ Microbiol*, 18(1): 232-245. <https://doi.org/10.1111/1462-2920.13005>
- Hamdali, H.,** Smirnov, A., Esnault, C., Ouhdouch, Y. & Virolle, M. J. (2010). Physiological studies and comparative analysis of rock phosphate solubilization abilities of Actinomycetales originating from Moroccan phosphate mines and of *Streptomyces lividans*. *Appl Soil Ecol*, 44(1): 24-31. <https://doi.org/https://doi.org/10.1016/j.apsoil.2009.09.001>
- Hanna, A. L.,** Youssef, H. H., Amer, W. M., Monib, M., Fayez, M. & Hegazi, N. A. (2013). Diversity of bacteria nesting the plant cover of north Sinai deserts, Egypt. *Journal of advanced research*, 4(1): 13-26. <https://doi.org/10.1016/j.jare.2011.11.003>
- Hewedy, M. A.,** Rushdy, A. A. & Kamal, N. M. (2013). Bioremediation of Rare Earth Elements and Uranium from Sinai soil, Egypt using actinomycetes. *Egypt J Hosp Med*, 53: 909-917. <https://doi.org/10.12816/0001653>
- Humber, R. A. (1997).** Chapter V-1 - Fungi: Identification. In: L. A. Lacey (Ed.), *Manual of Techniques in Insect Pathology* (pp. 153-185). London: Academic Press.
- Khandan Dezfally, N. & Gottravalli Ramanayaka, J. (2015).** Isolation, Identification and Evaluation of Antimicrobial Activity of *Streptomyces flavogriseus*, strain ACTK2 from Soil Sample of Kodagu, Karnataka State (India). *Jundishapur journal of microbiology*, 8(2): e15107. <https://doi.org/10.5812/jjm.15107>

- Klich, M. A. (2002).** *Identification of Common Aspergillus Species*. Utrecht-Germany: Centraalbureau Voor Schimmelcultures (pp. 1-116).
- Kora, M. & Jux, U. (1986).** On the Early Carboniferous macrofauna from the Um Bogma Formation, Sinai. *Neu Jb Geol Paläont, Mh*, 2: 85-98.
- Li, Q., Zhang, B., He, Z. & Yang, X. (2016).** Distribution and diversity of bacteria and fungi colonization in stone monuments analyzed by high-throughput sequencing, Brusetti, L. (ed). *PLoS One*, 11(9): 1-17. <https://doi.org/10.1371/journal.pone.0163287>
- Madigan, M. T., Martinko, J. M., Parker, J. & Brock, T. D. (1997).** *Brock Biology of Microorganisms* (8th ed.). New Jersey: Prentice-Hall International Inc. (pp. 440-442).
- Mohamed, S. H., Abdel-Fattah, H. I., Selim, S. M. & Sharaf, M. S. (2001).** Identification and molecular studies on some halotolerant streptomycetes isolated from Sinai sandy soil. *Arab Journal of Biotechnology*, 4: 179-196.
- Obuekwe, I. S. & Semple, K. T. (2011).** Impact of metals on the biodegradation of polycyclic aromatic hydrocarbons in soil. *Royal Society of Chemistry-Environmental Chemistry Group-Bull*, 7-10.
- Othman, A., Shawky, M., Amer, W., Fayez, M., Monib, M. & Hegazi, N. (2003).** Biodiversity of microorganisms in semi-arid soils of north sinai deserts. *Archives of Agronomy and Soil Science*, 49(3): 241-260. <https://doi.org/10.1080/0365034031000112083>
- Roushdi, M., Mostafa, H. & Kheireldin, K. (2016).** Present and Future Climate Extreme Indices over Sinai Peninsula, Egypt. *International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering*, 10: 84-90.
- Saadoun, I. & Gharaibeh, R. (2003).** The Streptomyces flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. *Journal of Arid Environments*, 53(3): 365-371. <https://doi.org/10.1006/jare.2002.1043>
- Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H. W., Perrone, G., Seifert, K. A., Susca, A., Tanney, J. B., Varga, J., Kocsubé, S., Szigeti, G., Yaguchi, T. & Frisvad, J. C. (2014).** Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in mycology*, 78: 141-173. <https://doi.org/10.1016/j.simyco.2014.07.004>
- Santhiya D., Ting Y.P. (2006).** Use of adapted *Aspergillus niger* in the bioleaching of spent refinery processing catalyst. *Journal of Biotechnology*, 121: 62-74.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol*, 16: 313-340. <https://doi.org/10.1099/00207713-16-3-313>

- Siddiqui, M. H.,** Kumar, A., Kesari, K. K. & Arif, J. M. (2009). Biomining - A Useful Approach Toward Metal Extraction. *Am-Eurasian J AGRON*, 2(2): 84-88. Retrieved from: [https://idosi.org/aeja/2\(2\)09/6.pdf](https://idosi.org/aeja/2(2)09/6.pdf)
- Thosar, A.,** Satpathy, P., T. N. & Rajan, A. P. (2014). Bio-mining: A revolutionizing technology for a safer and greener environment. *International Journal of Recent Scientific Research*, 5(9): 1624-1632.
- Valan Arasu, M.,** Duraipandiyan, V., Agastian, P. & Ignacimuthu, S. (2009). In vitro antimicrobial activity of *Streptomyces* spp. ERI-3 isolated from Western Ghats rock soil (India). *Journal de Mycologie Médicale*, 19(1): 22-28. <https://doi.org/10.1016/j.mycmed.2008.12.002>
- Watanabe, T. (2002).** *Pictorial atlas of soil and seed fungi : Morphologies of cultured fungi and key to species* (2nd ed.): CRC Press LLC.
- Whitman, W. B. (2012).** The Actinobacteria, Part A and B. In: M. Goodfellow, P. Kämpfer, H.-J. Busse, M. E. Trujillo, K.-i. Suzuki, W. Ludwig & W. B. Whitman (Eds.), *Bergey's manual of systematic bacteriology* (Second ed., Vol. 5). New York: Springer.
- Xu, L. H.,** Li, W. J., Liu, Z. H. & Jiang, C. L. (2007). *Actinomycete Systematics-Principle, Methods and Practice* (1st ed.). Beijing: Science Press.

الوصف الظاهري لبعض الأكتينوبكتيريا والفطريات المعزولة من الأسطح الصخرية المكشوفة في جنوب غرب سيناء بمصر

نهى محمد كمال^١، شيماء محمد عبد السلام^١، ناريمان مصلح حربى^٢، مها أمين هويدي^١، إبراهيم القطنى العاصى^٢.

- ١ قسم النبات - كلية النبات للآداب والعلوم والتربية - جامعة عين شمس - القاهرة - مصر - الرقم البريدى ١١٧٥٧.
- ٢ قسم الاستكشاف الجيوكيميائى - هيئة المواد النووية - القطامية - القاهرة - مصر - الرقم البريدى ١١٩٣٦.

المستخلص

لا يزال البحث عن سلالات جديدة ذو أهمية كبيرة في الأبحاث حول العالم من أجل التطبيقات الصيدلانية والصناعية والزراعية والتعدين باستخدام الكائنات الدقيقة. هذه الدراسة تهدف لدراسة التنوع الميكروبي لعينات صخرية تم جمعها من تكوين أم بجمة بجنوب غرب سيناء فى مصر والتي تم اختيارها نظرا لموقعها الفريد وخواصها الجيولوجية والفيزيوكيميائية الفريدة. أظهرت العينات التي تم دراستها تنوع ميكروبي قليل وعدد صغير من الميكروبات. تم عزل وعمل وصف ظاهري لمجموعة مكونة من عشرة عزلات من الأكتينوبكتيريا و عشرة عزلات من الفطريات. أشارت النتائج إلى أن جميع الأكتينوبكتيريا التي تم عزلها تنتمي إلى جنس *Streptomyces*. كانت جميعها من النوع المقاوم للملوحة وأظهر بعضها خصائص مضادة للميكروبات عند اختبارها ضد *Bacillus subtilis* ، *Pseudomonas aeruginosa* ATCC 9027 ، *Staphylococcus aureus* ATCC 6538 ، *Escherichia coli* ATCC 8739 ، *Candida albicans* و *Aspergillus flavus*. أما عن عزلات الفطريات التي تم دراستها فكانت جميعا تنتمي إلى جنس *Aspergillus*. وقد أظهرت مقاومة لمادة النستاتين المقاومة للفطريات عند استخدام تركيز ٥٠ ميكروجرام/مل. تتميز المناطق التي تم دراستها بظروفها القاسية والتي لا تدعم نمو معظم الكائنات الدقيقة ومنها درجة الحرارة التي تتراوح بين أقل من صفر درجة مئوية الى أكثر من ٤٦ درجة مئوية على مدار اليوم بجانب المحتوى المائى المنخفض وأيضا المادة العضوية المحتوية داخلها على عناصر مشعة وثقيلة. إن المجتمعات الميكروبية التي تعيش فى الصخور والتي نجت رغم هذه الظروف تفتح تحقيقات بحثية إضافية حول دراسة العلاقات التطورية بين هذه العزلات بالإضافة إلى الأنشطة الميكروبية المحتملة والتي يمكن ان تكون ذات أهمية بيئية أو صناعية.