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Evaluation of cold-adapted lipolytic activity of some Cladosporium, Penicillium, and Talaromyces species

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ABSTRACT

In this study, 216 isolates—104 *Cladosporium*, 94 *Penicillium*, and 18 *Talaromyces*—were evaluated for lipolytic activity at 10 °C on agar medium. Of the total isolates, 167 (77 *Cladosporium*, 81 *Penicillium*, and 9 *Talaromyces*) yielded positive results, accounting for 77.31 % of total. Of these, 40 isolates (14 *Cladosporium*, 25 *Penicillium*, and 1 *Talaromyces*) demonstrated high lipase activity, 54 moderate (25 *Cladosporium*, 22 *Penicillium*, and 7 *Talaromyces*), and 73 isolates (38 *Cladosporium*, 34 *Penicillium*, and 1 *Talaromyces*) displayed low lipolytic activity. All forty of the isolates from the first screening that had significant lipase activity at 10 °C were selected for the secondary screening under submerged fermentation conditions (SmF). Although in varying amounts, every isolate tested in the submerged fermentation secondary screening experiment was able to produce lipase. At 5 ºC, lipase's specific activity ranged from 6.1 to 353.4 U/mg protein; at 10 ºC, it was 3.83 to 353.34 U/mg; at 15 ºC, it was 6.77 to 463.75 U/mg; and at 20 ºC, it was 4.38 to 485.84 U/mg. Based on sequencing of internal transcribed spacer region (ITS), the two most active strains were identified as *Cladosporium cladosporioides* AUMC 15908 and *Talaromyces pinophilus* AUMC 308.

INTRODUCTION

The vast bulk of our world is covered by a variety of cold habitats, both natural and man-made [1]. In these environments, a diverse spectrum of psychrophilic microorganisms thrive, including archaea, bacteria, fungi, yeasts, and viruses, which are further classified as psychrophiles, or cold-loving microorganisms, and psychotropic, or cold-tolerant microorganisms [2]. Cold-adapted microbes, can be found in virtually any habitat, and they can be distinguished from mesophiles by their ability to thrive in temperatures below freezing [3]. The variety of microorganisms that can survive in hostile settings has been extensively studied.

Psychrophiles are among the most neglected sources of cold-adapted enzymes that are expelled and have high specific activity at low temperatures. Enzymes are gradually replacing chemical processes in industry because they are more environmentally friendly, boost product value, generate less waste, need less energy, and reduce environmental contamination [4-9].

Cold-adapted enzymes can catalyze at temperatures as low as 30 °C and retain some catalytic activity at temperatures as low as 0 °C [10]. They are distinguished from mesophilic and thermophilic enzymes by: (1) The low reaction energy, with the ideal catalytic temperature typically ranging from 20 to 45 °C; (2) higher substrate affinity which can lower the activation energy of the enzymatic reactions; (3) low thermal stability at high temperatures, and quickly loss of more than half of their activity after 10 minutes at 50-60 °C or several hours at 37 °C [11-16].

Cold-adapted enzymes from microbes have the benefits of abundant sources, short production cycle, high yield, and facile separation and purification, and hence have garnered a lot of attention [17-19]. Cold-adapted enzymes offer numerous industrial applications, including easily controlled reaction conditions, convenient monitoring and control of production processes, and a wide range of commercial applications. As a result, these enzymes have been thoroughly explored and used in a number of applications, such as food processing, detergent manufacturing, bioremediation, environmental protection, straw resourcing, and basic molecular biology research [20, 21].

Lipases (triacylglycerol hydrolases; EC 3.1.1.3) are co-factor-independent enzymes of high economic importance. They are widely used in the manufacturing of fine chemicals, as well as the food, textile, pulp and paper, laundry, and biodiesel sectors. Microbial lipases are popular in commercial applications due to their diverse enzymatic characteristics and substrate selectivity [22]. Lipases are one of the most popular types of enzymes in terms of overall sales. The global market for microbial lipase was valued at USD 349.8 million in 2019 and is estimated to rise at a compound annual growth rate (CAGR) of 5.2 % between 2020 and 2025, reaching USD 428.6 million. By 2025, the global lipase market is estimated to generate more than USD 797.7 million [23].

According to recent research, microorganisms produce the vast majority of enzymes used in industrial processes nowadays, making the microbial lipase market one of the most dynamic industry verticals. Purified lipases are required for the biocatalytic manufacturing of products in

all of the aforementioned industries [24]. Microbial lipases are particularly useful in industry due to their resilience to temperature and pH extremes, as well as their broad substrate specificity [25, 26]. Lipases are present throughout nature and are active at a variety of temperatures. Cold active lipases function at temperatures ranging from 0 to 30 °C [27]. Therefore, the goal of the current investigation was to evaluate the activity of cold-adapted lipase for some *Cladosporium*, *Penicillium*, and *Talaromyces* species both on agar media as well as in submerged fermentation (SmF).

MATERIALS AND METHODS

Screening the lipolytic activity

Fungal species

Two hundred and sixteen isolates were included in this test. These were 104 isolates related to *Cladosporium* (9 identified isolates that belonging to three species, and 95 unidentified isolates), 94 *Penicillium* (62 identified represented by 17 species and 32 unidentified), and 18 *Talaromyces* (represented by 6 species). All isolates in this test were retrieved from the culture collection of the Assiut University Mycological Centre (AUMC).

Lipase production medium

Lipase production medium described by Ullman and Blasins [28] was used. The medium contained (g/L): peptone, 10; $MgSO_4$.$7H_2O$, 0.2; $CaCI_2.2H_2O$, 0.2; Tween 80, 10 mL; and agar, 15). The medium was sterilized by autoclaving at 121°C for 15 minutes. The Tween 80 was autoclaved separately and added to the sterile and cooled basal medium.

Preliminary screening of lipolytic activity

Test tubes measuring 15 cm were used to aseptically dispense the medium (12 mL/tube). A 50 µL spore suspension obtained from 7-day-old fungal cultures were used to separately inoculate the test tubes on the agar surface, and the tubes were then incubated for 7, 14, and 21 days at 10 °C. The creation of calcium salt crystals from the oleic acid that the enzyme released was a clear indicator of the fungus's lipolytic capabilities. Each observable precipitate's depth (measured in millimeters) was determined. The positive fungal lipase producers were categorized, according to the precipitate depth, to low, moderate, and high.

Secondary screening of lipolytic activity

Secondary screening was performed for the highest lipase-producing isolates from the prior experiment. Sucrose-free Cz broth [29] supplemented with 1.0 % Tween 80 as a sole carbon source was used as the fermentation medium. Each 250-mL Erlenmeyer conical flask containing 50 mL of fermentation medium had been separately inoculated with 1.0 % (v/v) spore suspension containing 1.5×10^8 spore/mL derived from a 7-day-old culture of the tested fungi. The flasks were subsequently incubated at 10 °C for 7 days under agitated conditions of 150 rpm.

Extraction and assay of the cold-active lipase and total protein estimation

The cell-free supernatant was recovered by centrifuging at 10,000 rpm for 10 minutes at 4 °C after the fermentation period, and this was utilized as the source of the cold-active lipase. In order to measure the lipolytic activity quantitatively, 3 mL of 1.0 % Tween 80 were mixed with 2.5 mL of deionized water and 1.0 mL of the appropriate buffer solution for the pH that was being evaluated. At 5, 10, 15, and 20 °C, the mixture and the enzyme were kept apart. The reaction was then started for 60 minutes by adding 1.0 mL of the supernatant to the mixture. After adding 3 mL of 96 % ethyl alcohol to stop the reaction, the mixture was titrated against a 0.05 M NaOH solution with 4 drops of phenolphthalein solution (0.1 g diluted in 50 mL of 1: 1 ethanol: distilled water) until pink coloration appeared. Each buffer solution that was utilized underwent titration, and the lipase activity was calculated using the volume of NaOH for both the buffer solution (V0) and the sample (V1). Equation (1) was utilized to compute the lipase activity.

$$\textbf{Lipase activity} = \frac{V1 - V0 \times M \times 1000}{V2 \times T} \quad \textbf{U/mL} \qquad \textbf{(1)}$$

Where: V1 = Volume of NaOH (mL) consumed for a sample; V0 = Volume of NaOH consumed for the blank; M = Molarity of NaOH (mM); 1000 = Conversion factor from milli-equivalent to micro-equivalent; DF = Dilution factor of the enzyme; V2 = Volume of lipase used in the reaction; and T = Time of the reaction (min).

Selection and preservation of the potent strains

The most potent isolate from each genera was selected. Two isolates belonging to *Cladosporium* and *Talaromyces* (anamorph: *Penicillium*), which were recorded as the potent fungi, were selected for molecular identification. Pure cultures of the *Cladosporium* and

Talaromyces isolates were preserved at the Assiut University Mycological Centre (AUMC), Assiut, Egypt, and were given the number AUMC 1908 and AUMC 308, respectively.

Morphological identification of the *Cladosporium* and *Talaromyces* isolates

The *Cladosporium* isolate was morphologically identified by cultivating the fungus on Petri plates containing potato dextrose agar (PDA), oat agar (OA), and synthetic nutrient agar (SNA), while *Talaromyces* isolate was cultivated on Czapek's-Dox agar (CzA), malt extract agar (MEA), and Czapek's Yeast Autolysate agar (CYA) [29]. The cultures were then incubated at 25 °C for 7 days [30]. This allowed for the examination of macroscopic and microscopic fungal features.

Molecular identification of the *Cladosporium* and *Talaromyces* isolates

For DNA isolation, the method described by Moubasher *et al.* [31] was followed. PCR reaction was performed using SolGent EF-Taq polymerase [32, 33]. The universal primers ITS1 and ITS4 were used for ITS region amplification [34]. Contiguous sequences of the *Talaromyces* and *Cladosporium* isolates used in this study were produced using DNASTAR (version 5.05). The most similar sequences to the fungi utilized in this study were downloaded from the GenBank database. MAFFT (version 6.861b) was used in this investigation to align all sequences using the default parameters [35]. Alignment gaps and parsimony uninformative characters were optimized by BMGE [36]. MEGA X (version 10.2.6) was used to conduct maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic analyses [37], and the robustness of the most parsimonious trees was evaluated by 1000 replications [38]. Utilizing Modeltest 3.7's Akaike Information Criterion (AIC), the optimum nucleotide substitution model for ML analysis was identified [39].

RESULTS

Preliminary screening the lipolytic activity of the fungal isolates

The preliminary screening results of the 216 isolates tested at 10 °C revealed that, 167 isolates (77 *Cladosporium*, 81 *Penicillium* and 9 *Talaromyces*), comprising 77.31% of total isolates, exhibited positive results, of which 40 isolates (14 *Cladosporium*, 25 *Penicillium* and one *Talaromyces*) had high lipase activity, 54 demonstrated moderate activities (25

Cladosporium, 22 *Penicillium* and 7 *Talaromyces*), and 73 isolates (38 *Cladosporium*, 34 *Penicillium* and one *Talaromyces*) displayed low lipolytic activity (Table 1; Figure 1).

Table 1. Preliminary screening of the cold-adapted lipolytic activity of some *Penicillium* species on sucrose-free Cz amended with 1.0 % Tween 80 at 10 °C.

Fungal species	No. of isolates	Positive isolates	Preliminary screening		
			H	M	L
Cladosporium	١٠٤	٧7	١٤	٢5	٣٨
C. cladosporioides	٦	٣	3	0	0
C. herbarum	١	٠	0	0	0
C. sphaerospermum	٢	٢	1	1	0
Cladosporium spp.	٩٥	٧2	10	24	38
Penicillium	**94**	**81**	**25**	**22**	**34**
P. aurantiogriseum	7	7	4	3	
P. brevicompactum	1	1	1		
P. camemberti	1	0			
P. canescens	1	1			1
P. chrysogenum	8	8	2	3	3
P. citrinum	6	2			2
P. corylophilum	2	1			1
P. crustosum	9	6	2		4
P. expansum	5	5	2	3	
P. griseofulvum	5	5	5		
P. italicum	3	3		1	2
P. simplicissimum	1	1	1		
P. jensenii	1	1	1		
P. janczewskii	2	2	1	1	
P. oxalicum	6	6	1	2	3
P. polonicum	1	1			1
P. roquefortii	3	3		2	1
Penicillium spp.	32	28	5	7	16
Talaromyces	**18**	**9**	**1**	**7**	**1**
T. duclauxii	5	0			
T. funiculosus	5	4		3	1
T. islandicus	1	1		1	
T. pinophilus	1	1	1		
T. purpureogenus	4	2		2	
T. variabilis	2	1		1	
Total	**216**	**167**	**40**	**54**	**73**

Preliminary screening: H = ≥ 10 mm; M = 6 to 9 mm; L = ≤ 5 mm

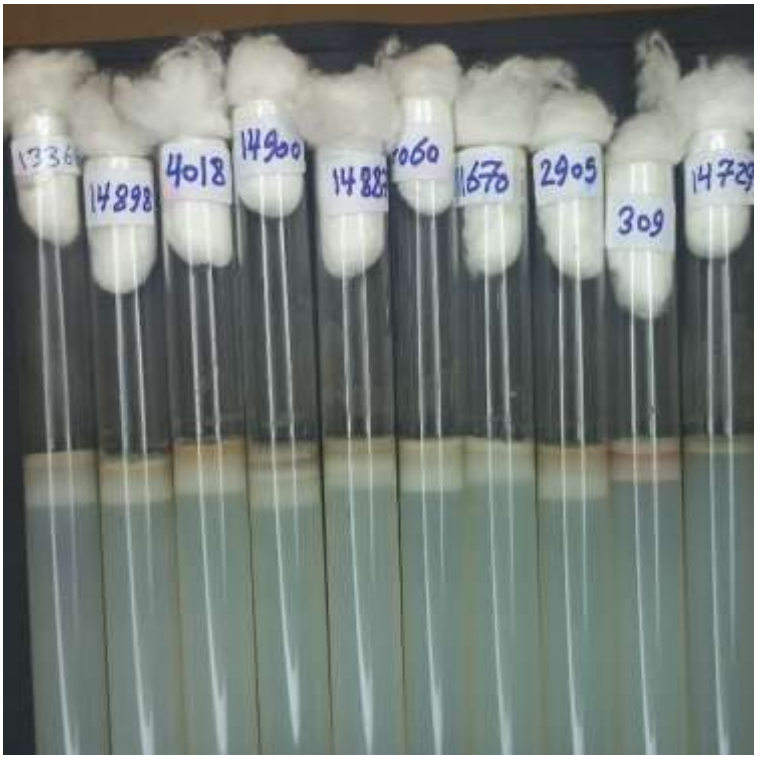

Figure 1. Lipolytic activity shown as calcium oleate precipitate underneath fungal growth

For the secondary screening, which was conducted under submerged fermentation conditions (SmF), all the forty isolates from the initial screening that showed appreciable lipase activity at 10 °C were chosen. All isolates examined in the secondary screening experiment using submerged fermentation were able to produce lipase, albeit in different quantities. The specific activity of lipase varied from 6.1 to 353.4 U/mg protein at 5 ºC, 3.83 to 353.34 U/mg at 10 ºC, 6.77 to 463.75 U/mg at 15 ºC, and 4.38 to 485.84 U/mg at 20 ºC. It has been shown that twenty-one isolates produce substantial levels of lipase; the two most potent strains were related to isolates of *Cladosporium* and *Talaromyces* (Table 2).

Table 2. Determination of the lipolytic relative activity (U/mL) and specific activity (U/mg) at different temperatures of the high lipase-producing fungi.

Fungal species	AUMC No.	Source	Lipase relative activity (U/mL)				Lipase specific activity (U/mg)			
			5 ºC	10 ºC	15 ºC	20 ºC	5 ºC	10 ºC	15 ºC	20 ºC
C. cladosporioides	**١٥٩٠٨**	**Air, cold room**	**1.42**	**1.5**	**1.59**	**2.25**	**123.1**	**124.39**	**125.75**	**136.58**
C. cladosporioides	11177	Maize	1.17	1.25	1.67	1.59	9.3	9.89	13.19	12.53
C. cladosporioides	11193	Maize	1.17	1.25	1.59	1.17	9.7	10.29	13.04	9.61
C. sphaerospermum	11172	Maize	1.25	1.0	1.59	1.67	9.9	3.88	7.81	9.79
C. sphaerospermum	11366	Air	1.25	0.67	1.25	0.84	13.9	7.4	13.86	9.24
Cladosporium sp.	AMO-5	Air, cold room	1.34	1.0	1.25	1.17	7.3	5.41	6.77	6.31
Cladosporium sp.	AMO-12	Air, cold room	1.67	1.09	1.5	0.0	25.6	16.6	22.98	0.0
Cladosporium sp.	AMO-20	Air, cold room	1.34	0.75	1.25	1.0	8.6	4.79	7.99	6.39
Cladosporium sp.	AMO-29	Air, cold room	0.92	0.59	1.09	0.67	6.1	3.83	7.11	4.38
Cladosporium sp.	AMO-40	Air, cold room	1.25	1.42	1.25	1.0	8.4	9.46	8.35	6.68
Cladosporium sp.	AMO-42	Air, cold room	1.34	0.59	1.34	0.84	9.7	4.23	9.66	6.04
Cladosporium sp.	AMO-50	Air, cold room	1.0	0.42	1.0	0.75	10.4	4.34	10.4	7.8
Cladosporium sp.	AMO-70	Air, cold room	0.75	1.0	1.09	1.0	9.0	12.0	13.0	12.0
Cladosporium sp.	AMO-97	Air, cold room	0.92	1.0	1.34	1.0	9.3	10.12	13.49	10.12
P. aurantiogriseum	513	Sweet potatoes	1.34	1.17	1.59	1.84	15.1	13.16	17.86	20.68
P. aurantiogriseum	344	Soil	1.17	1.0	1.5	1.84	12.1	10.36	15.53	18.98
P. aurantiogriseum	347	Soil	1.34	1.09	1.67	1.84	33.1	26.84	41.28	45.41
P. aurantiogriseum	526	Soil	1.59	1.42	1.67	1.75	18.7	16.69	19.63	20.62
P. brevicompactum	528	Cereal aphids	1.5	1.09	1.67	1.67	39.0	28.15	43.31	43.31
P. chrysogenum	14554	Lemon fruit	1.5	1.75	1.84	2.42	16.9	19.66	20.59	27.14
P. crustosum	505	Sweet potatoes	1.42	1.17	1.59	1.67	25.6	21.04	28.55	30.05
P. crustosum	15155	Unknown	1.42	1.42	1.67	1.5	10.0	9.94	11.69	10.52
P. echinulatum	584	Reservoir water	1.34	1.42	1.84	1.84	15.9	16.84	21.79	21.79
P. expansum	515	Air	1.5	1.0	1.75	2.0	18.0	11.94	20.89	23.88
P. griseofulvum	309	Soil	1.42	1.42	1.59	1.84	15.4	15.39	17.2	19.92
P. griseofulvum	503	Soil	1.5	0.92	1.67	1.84	12.1	7.39	13.43	14.77
P. griseofulvum	504	Soil	1.42	1.25	1.59	1.84	78.3	69.02	87.42	101.22
P. griseofulvum	534	Soil	1.25	1.5	1.92	2.17	17.9	21.38	27.31	30.87
P. griseofulvum	535	Soil	1.59	1.34	1.59	1.84	17.5	14.67	17.42	20.16
P. janthinellum	529	Soil	1.67	1.42	1.75	2.0	12.1	10.26	12.68	14.49
P. jensenii	320	Soil	1.34	1.25	1.59	1.92	16.7	15.63	19.8	23.96
P. nigricans	315	Soil	1.25	1.0	1.5	1.67	25.5	20.39	30.58	33.98
P. oxalicum	322	Soil	1.42	1.5	1.34	1.84	15.1	15.9	14.14	19.44
Penicillium sp.	502	Soil	1.42	1.59	1.67	2.59	13.6	15.15	15.95	24.72

Fungal species	**AUMC No.**	**Source**	**Lipase relative activity (U/mL)**				**Lipase specific activity (U/mg)**			
Penicillium sp.	580	Reservoir water	1.59	1.34	1.59	2.25	16.4	13.75	16.33	23.21
Penicillium sp.	583	Reservoir water	1.5	1.5	1.59	1.84	18.3	18.24	19.25	22.29
Penicillium sp.	4034	Air, Saudi Arabia	1.59	1.42	1.84	1.75	82.3	73.62	95.27	90.94
Penicillium sp.	4050	Air, Saudi Arabia	1.59	1.59	2.34	1.84	14.7	14.68	21.63	16.99
Penicillium sp.	AMO-11	Air, cold room	1.67	1.42	1.84	1.84	14.9	12.65	16.36	16.36
Talaromyces pinophilus	**308**	**Soil**	**1.34**	**1.34**	**1.75**	**1.84**	**353.4**	**353.34**	**463.75**	**485.84**

Morphological identification of the potent isolates

Cladosporium cladosporioides AUMC 15908

Microscopic characteristics. Conidiophores solitary, macronematous, arising terminally or laterally from hyphae, straight to somewhat flexuous, non-nodulose, 40-300 (-350) × (2.5-) 3-4 (-5.5) µm, unbranched or occasionally branched, branches usually short, only as peg-like lateral outgrowth just below a septum. Conidiogenous cells integrated, usually terminal, sometimes intercalary with conidiogenous loci situated on denticle-like lateral outgrowths, cylindrical, not geniculate, non-nodulose, (7-)16-38 µm long, with up to four loci crowded at the apex. Ramoconidia straight to slightly curved, cylindrical, 15-50 × (2.5-)3-5 µm, with up to three septa, pale olivaceous-brown, smooth, 2.5-4 µm wide, unthickened or slightly thickened. Conidia abundant, catenate, aseptate, in long branched chains, up to 10 conidia in the upper unbranched part, branching in all directions, small terminal conidia subglobose, obovoid, ovoid to limoniform, 3-6(-7) × (1.5-)2-2.5(-3) µm (Figure 2).

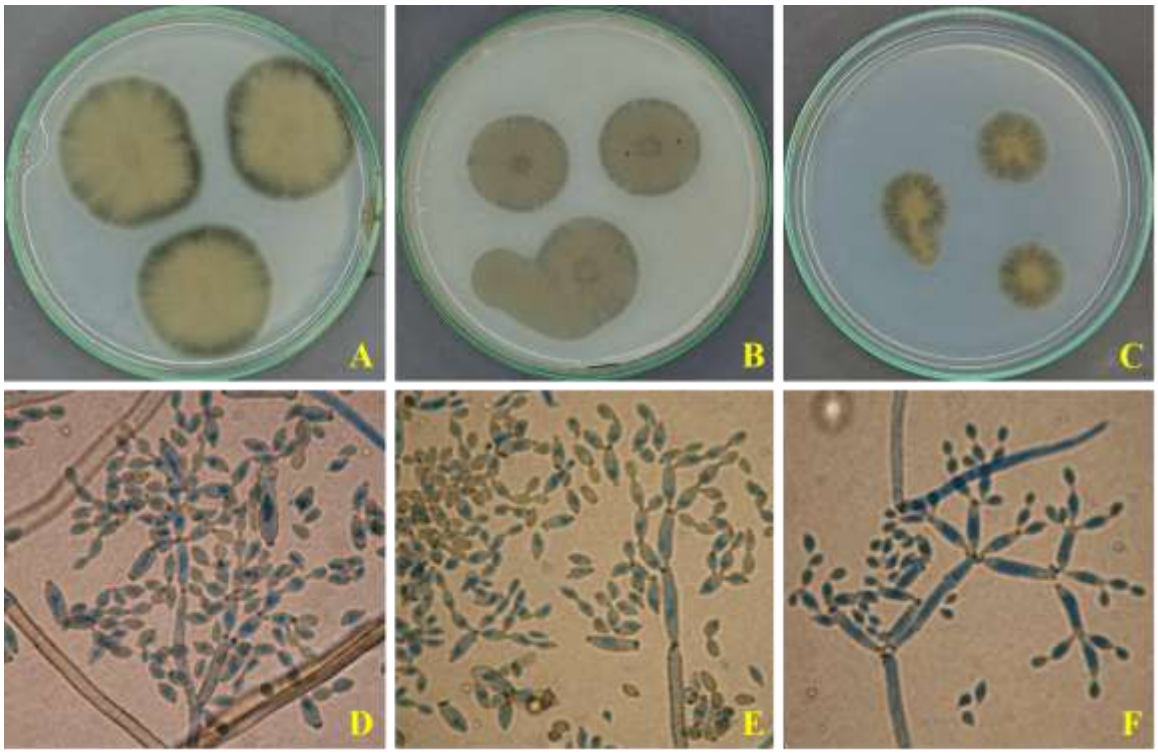

Figure 2. *Cladosporium cladosporioides* AUMC 15908. (A – C) Seven-day-old cultures on PDA, OA, and SNA at 25 C. (D – F) Conidiophores, ramiconidia, and smooth conidia

Talaromyces pinophilus AUMC 308

Microscopic characteristics. Morphologically, the *Talaromyces* strain in this study showed the identical characteristics of *Talaromyces pinophilus* as fast-growing colonies (48–55 mm) with distinctly funiculose surface, yellow margin and yellow-green conidial areas. Conidiophore long, commonly up to 130 µm. Metulae appressed, 10–13 µm. Phialides typically lanceolate, closely appressed, 7–10 µm. Conidia subglobose to elliptical, 2–3 × 2–2.5 µm (Figure 3).

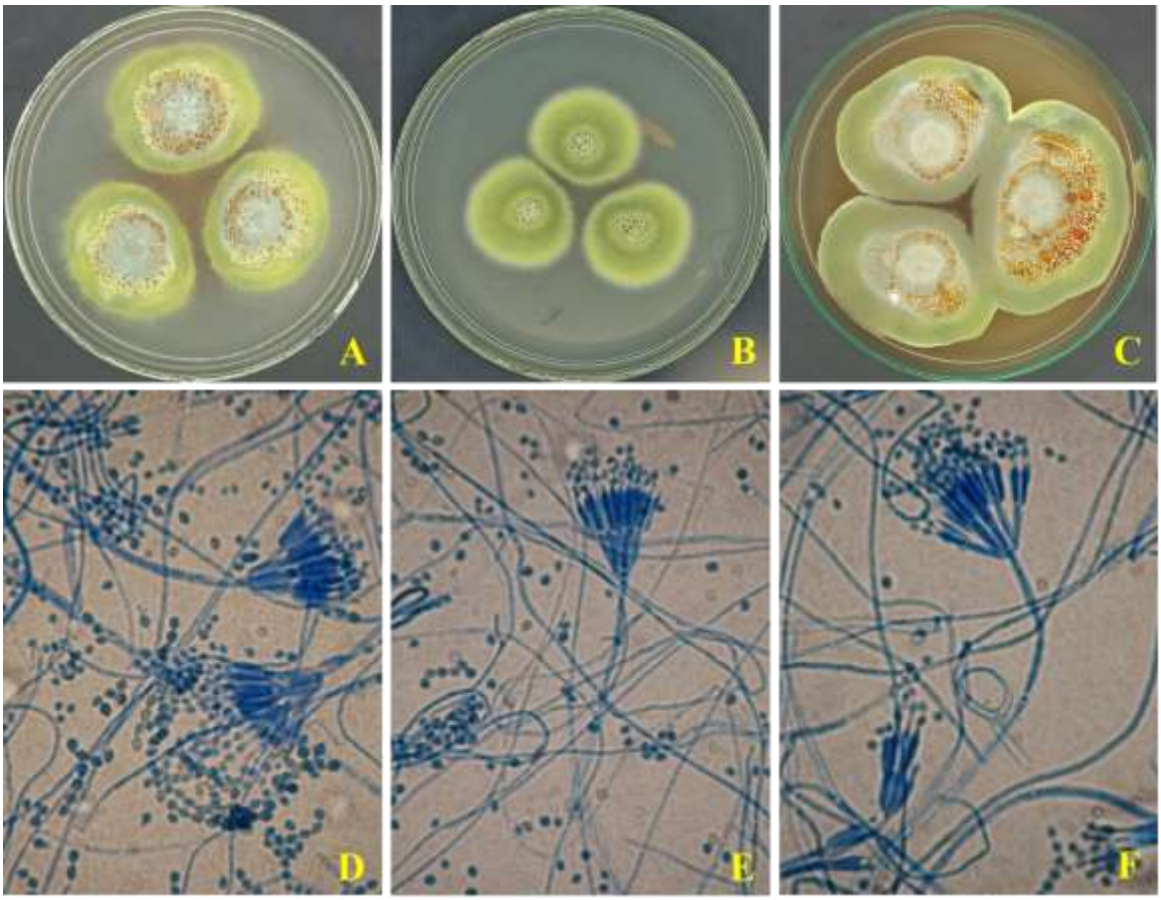

Figure 3. *Talaromyces pinophilus* AUMC 308. (A–C) Seven-day-old colonies on Cz, MEA, and CYA at 25 ºC. (D – F) Conidiophores, penicilli, and conidia.

Molecular confirmation of the potent isolates

Cladosporium cladosporioides AUMC 15908

A megablast search in NCBI database using ITS sequence of the *Cladosporium* isolate AUMC 15908 in this study revealed that, the closely similar hits are *Cladosporium cladosporioides* isolates CEL14 and BAB-6501 [(GenBank accession

numbers MN173120 and MF319920, respectively; identities = 523/523 (100%); Gaps = 0/523 (0%)]. The entire ITS dataset comprised 12 sequences. The maximum parsimony dataset consisted of 564 characters of which 512 characters could aligned unambiguously, 170 variable characters which were parsimony-uninformative, and 2 characters were counted as parsimony informative. Kimura 2-parameter (K2) was the perfect model for nucleotide substitution. Maximum parsimony analysis resulted in 5 trees, the most parsimonious of which has tree length of 201 steps, consistency index of 0.777778, retention index of 0.818182 and composite index of 0.636364 is shown in Figure 4. Maximum likelihood analysis yielded one tree with the highest log likelihood of -1464.58. The strain of *Cladosporium cladosporioides* AUMC 15908 in this study located at the *Cladosporium cladosporioides* clade with *Cladosporium cladosporioides* CEL14 and BFMY-2 confirming its identification as *Cladosporium cladosporioides* (Figure 4).

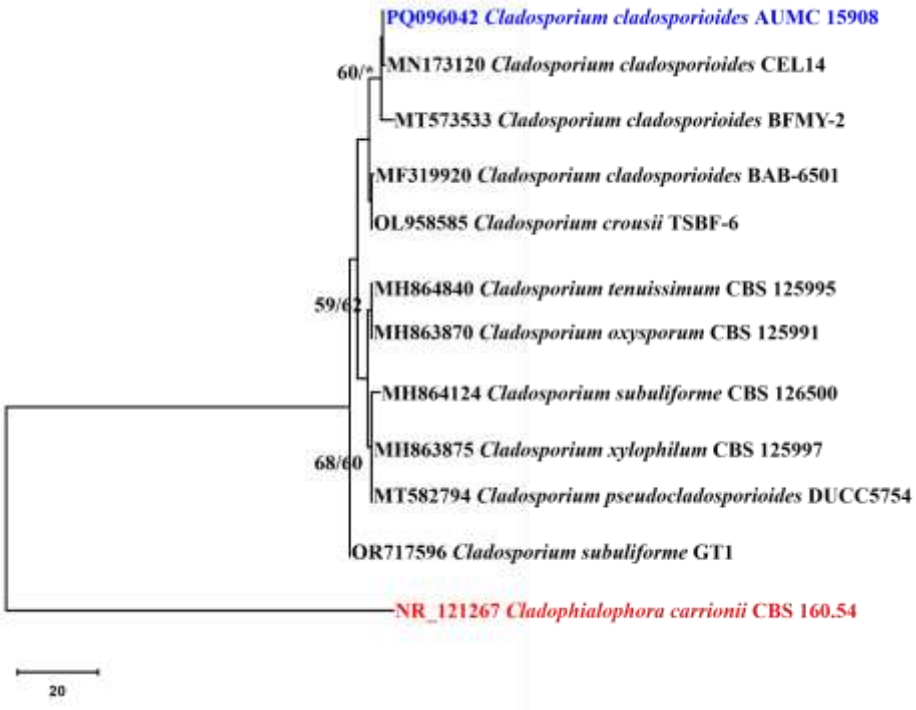

Figure 4. Evolutionary phylogenetic tree for ML/MP obtained from a heuristic search (1000 replications) of *Cladosporium cladosporioides* AUMC 15908 ITS sequence (in blue) compared to the closely similar ITS sequences belonging to *Cladosporium* in GenBank. Bootstrap support values for ML/MP ≥50% are indicated near the tree nodes. The tree is rooted to *Cladophialophora carrionii* CBS 160.54 as outgroup (in red).

Talaromyces pinophilus AUMC 308

A megablast search in NCBI database using ITS sequence of the *Talaromyces* isolate in this study revealed that, the closely similar hits are *Talaromyces pinophilus* strains KUMCC 18-0203, 17F4103, and RGM 2652 [(GenBank accession numbers MT152340, MT093464, and MN587879, respectively; identities = 552/552 (100%); Gaps = 0/552 (0%)]. The entire ITS dataset comprised 15 sequences. The maximum parsimony dataset consisted of 580 characters of which 523 characters could aligned unambiguously, 96 variable characters which were parsimony-uninformative, and 2 characters were counted as parsimony informative. Tamura 3-parameter with Gamma distribution (T92+G) was the perfect model for nucleotide substitution. Maximum parsimony analysis resulted in 9 trees, the most parsimonious of which has tree length of 118 steps, consistency index of 0.923077, retention index of 0.941176 and composite index of 0.876984 is shown in Figure 5. Maximum likelihood analysis yielded one tree with the highest log likelihood of -1334.52. The strain of *Talaromyces* sp. AUMC 308 in this study located at the *Talaromyces pinophilus* clade with *Talaromyces pinophilus* RGM_2652 endorsing strongly-supported clade (63% ML/99% MP).

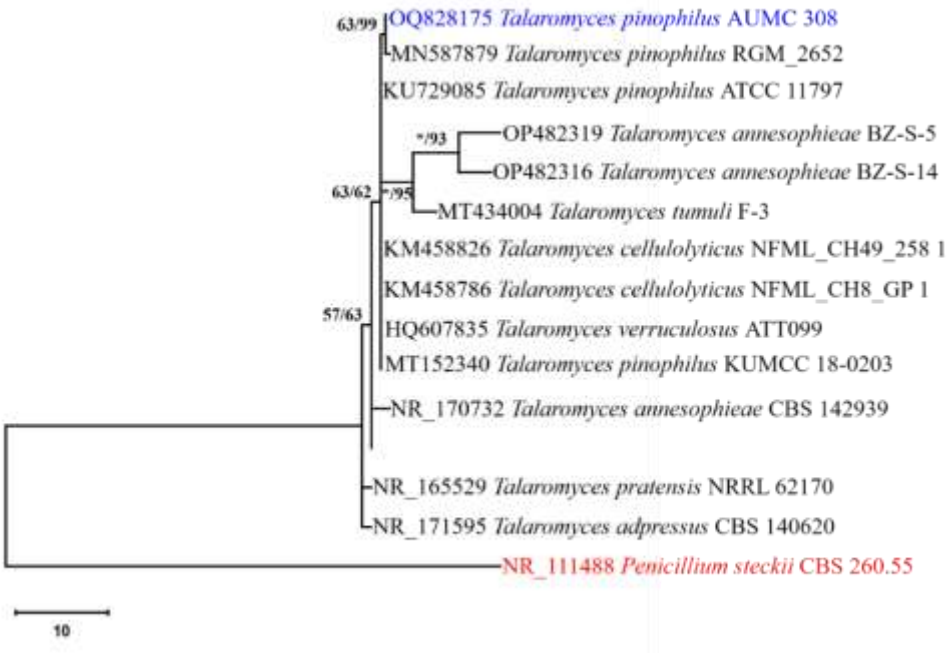

Figure 5. Evolutionary phylogenetic tree for ML/MP obtained from a heuristic search (1000 replications) of *Talaromyces pinophilus* AUMC 308 ITS sequence (in blue) compared to the closely similar ITS sequences belonging to *Talaromyces* in GenBank. Bootstrap support values for ML/MP ≥50% are indicated near the tree nodes. The tree is rooted to *Penicillium steckii* CBS 260.55 as outgroup (in red).

DISCUSSION

Lipases have piqued the interest of scientists and industrialists due to its applications in several economic sectors such as food, cosmetics, chemical industries, and medicines. As a result, a certain level of purity is required [40, 41]. The majority of the Earth's crust and atmosphere has permanently cold (5 °C) habitats that support psychrophilic microbes such as archaea bacteria, yeast, fungi, and algae [1]. These microorganisms' capacity to exist in such frigid circumstances necessitates many adaptation methods that allow them to proliferate and undertake metabolic activity at low temperatures [42, 43]. Cold-adapted enzymes (amylase, lipase, protease, etc.) are secreted by cold adapting microorganisms and are known to retain stability and activity under low temperature circumstances. These cold-tolerant enzymes have been linked to a variety of biotechnological applications [20, 44].

This study examined 216 isolates of *Cladosporium*, *Penicillium*, and *Talaromyces*, to determine their capacity to produce cold-adapted lipase at 10 °C. 76.85 % of the isolates (166 isolates) showed positive lipase activity during the preliminary screening of the lipolytic activity. Of them, 76 isolates of *Cladosporium* were found, 81 isolates of *Penicillium*, and 9 isolates of *Talaromyces* species made up the 45.78, 48.79, and 5.42 % of positive isolates. According to the screening findings in this study, it was found that the most productive strains were *Cladosporium cladosporioides* AUMC 15908 and *Talaromyces pinophilus* AUMC 308. In this regard, Numerous fungi, such as *Aspergillus*, *Candida*, *Mucor*, *Penicillium*, and *Rhizopus* have been reported for their extracellular lipases production [44-49]. Because these enzymes are inducible, their yield is determined by a variety of conditions.

İrdem *et al.* [50] examined 52 microfungi that were obtained from Turkey's Acıgöl Lake in order to determine their lipolytic activity. It was demonstrated that 92.0% of the isolates exhibited lipolytic activity. The three most active strains with notable lipolytic activity were *Aspergillus amstelodami*, *Penicillium sizovae*, and *Penicillium*

solitum. Kuncharoen *et al.* [51] tested thirty-three yeasts that were isolated from palm oil industrial wastes and traditional fermented foods in Thailand for their lipolytic potential. Seven strains, *Magnusiomyces capitatus* 5E-1T and 5E-2D, *Trichosporon asteroides* 8E-1T and 8E-1D, *Trichosporon insectorum* 4E-1D, and *Yarrowia lipolytica* Fy-12 and Fy-13, showed high lipolytic activity. Mahmoud *et al.* [52] conducted a screening of 134 fungal isolates that were isolated from sesame, peanut, and soybean collected from various stores within the Assiut Governorate. Out of these isolates, 39 shown lipolytic capabilities, with *Aspergillus niger* demonstrating the highest level of activity. Two distinct extracellular lipases were obtained from *Penicillium solitum* 194A, isolated from domestic compost, and *Cladosporium cladosporioides* 194B, isolated from dairy wastewater [53]. Four fungal species were recovered from water of the Caspian Sea (northern Iran). Among these organisms, *Cladosporium langeronii* showed the strongest lipolytic activity (34 U/mL) [54]. Using solid state fermentation of soybean bran and tributyrin on agar plates, five fungi out of 24 were found to be good lipase producers. These species were related to *Aspergillus* and *Penicillium* [26].

Sequencing of the ITS region was carried out to validate the identification of the two most active species in this study. The internal transcribed spacer (ITS) region, which lies between the 18S and 28S rRNA genes, has been used to classify several fungal species and is an area of special interest for differentiating between closely related or intraspecific species because it has high survival and variability in certain areas [31-33, 55-62].

In this study, all isolates examined in the secondary screening experiment using submerged fermentation could produce lipase, albeit in different quantities. The specific activity of lipase varied from 6.1 to 353.4 U/mg protein at 5 ºC, 3.83 to 353.34 U/mg at 10 ºC, 6.77 to 463.75 U/mg at 15 ºC, and 4.38 to 485.84 U/mg at 20 ºC. It has been shown that twenty-one isolates produce substantial levels of lipase; the two most potent strains were related to isolates of *Cladosporium* and *Talaromyces*. The design of the bioreactor, media composition, and culture practices (submerged or solid state fermentation) are the main factors influencing lipase production. The media requirements for effective lipase synthesis are principally determined by carbon, nitrogen, and lipid

supplies. Achieving high yield and maximum activity is crucial in numerous industrial areas. These factors have been taken into account when designing a number of studies on medium optimization for lipase production optimization. Therefore, to increase the quantity of lipase produced by the potent strains in the present investigation, the fermentation parameters (pH, nitrogen source, temperature, fermentation time, and lipid source) need to be optimized.

CONCLUSION

The cold-active lipolytic activity of 216 isolates associated with *Cladosporium*, *Penicillium*, and *Talaromyces* was assessed in this investigation. Forty of the isolates that produced positive results had significant lipase activity. All of the isolates examined in the subsequent screening experiment using submerged fermentation were able to produce lipase, albeit in different quantities. The specific activity of lipase varied from 6.1 to 353.4 U/mg protein at 5 ºC; 3.83 to 353.34 U/mg at 10 ºC; 6.77 to 463.75 U/mg at 15 ºC; and 4.38 to 485.84 U/mg at 20 ºC. The two most active strains were determined to be *Cladosporium cladosporioides* AUMC 15908 and *Talaromyces pinophilus* AUMC 308 based on internal transcribed spacer region (ITS) sequencing.

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