Libyan Government

Management of Postgraduate Studies and Training

University of Zawia – Faculty of Science

Biology Department – Botany Branch

Cold-active lipases produced by fungi isolated from the solid wastes (Fatura) of the olive oil industry in Al-Gabal Al-Gharby, Libya

A thesis submitted to the Botany Department, Faculty of Science, Zawia University, Libya

in partial fulfillment for Master degree in Science – Botany (Microbiology – Fungi)

Presented by

Joheni Mohamed Alhadi Jwely

Under Supervision of

Prof. Dr. Mohamed Ahmed Abulkasem Al-Ryani

Professor of Mycology

Department of Botany, Faculty of Science,

Zintan University

2023

DEDICATION

To the memory of my father and my uncle AL-Mabrouk, and sincere thanks and appreciation to my lovely mother.

Ishani M. Jively

ACKNOWLEDGEMENT

All the greatest gratefulness, deepest appreciation and thanks to **ALLAH** for the many things he gave me the patience, knowledge and for enabling me overcome all the problems which faced me in my life and finish this work.

I would like to express my sincere appreciation and deepest thank to ***Prof Dr; Mohamed A. AL-Ryani.*** Professor of Mycology, the Supervisor and Previous Head at the College of Medical Technology at Zintan University, for his kind advice and supervision continuous support, encouragement, useful criticism, and laborious guidance during the progress of this investigation, interest, and for his keen interest for every detail in this course of study.

I wish to express my sincere appreciation and my deepest thank to ***Dr. Osama AL-Bedak***, Assistant Professor of Mycology, Botany & Microbiology Department, Faculty of Science, Assiut University, for useful criticism and laborious guidance during the progress of this investigation.

I would like to express my deepest gratitude and appreciation to ***Prof Dr; Ahlam Rashid,*** Professor of Physiology, Head of the Botany Department, Zawia University and Head of Advanced Research Center for Botany and Medicine, for her kind advice, useful and continuous support and encouragement.

I would like to express my thanks and deep gratitude to ***Prof Dr; Ahmed M. Moharram***, Botany and Microbiology Department, Faculty of Science, Assiut University for his help in providing me with all the capabilities to accomplish throughout this work.

My thanks and gratitude's are also to all friends and members of the Mycological Center, Assiut University. Finally, sincere thanks and appreciation are also to my mother for her help, patience, assistance and moral support.

Johayna Mohamed

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION

$5H_2O$	Penta Hydrate
AIC	Akaike Information Criterion
AUMC	Assiut University Mycological Centre
BMGE	Block Mapping and Gathering with Entropy
BSA	Bovine Serum Albumin
Ca^{2+}	Calcium cation
Ca_2C	Calcium Carbide
$CaCl_2$	Calcium chloride
CFUs	Colony Forming Units
CO	Carbon Monorxide
CO_2	Carbon Dioxide
$CoCl_2$	Cobalt chloride
COD	Chemical Oxygen Demand
CTAB	Cetltrimethyl Ammonium Bromide
Cu^{2+}	Copper cation
$CuSO_4$	Copper Sulphate
$CuSO_4$	Copper sulfate
Cza	Czapek′s Agar
DCCR	District and Coordinator Resources
DDH_2O	Double-Distilled water
DF	Dilution Factor of enzyme
DNA	Deoxyribonucleic Acid
DVL2	Isolate Discovered in Ordinary City
EDTA	Ethylenediaminetetraacetic acid
$FeSO_4$	Iron Surfate
Fe^{2+}	Iron cation
$FeSO_4$	Iron sulfate
H_2O_2	Hydrogen Peroxide
ITS1	Internal Transcribed Spacer(ITSI)
KCl	Potassium Chloride
LED	Light Emitting Diode
LIPYS	*Yarrowia lippolytica*
M	Morality of Noah (mm):1000=conversion factor from milli-equivalent to micro-equivalent
MAFFT	Multiple Alignment program for amino acid or nucleotide sequences
MEGA	Molecular Evolutionary Genetics Analysis
Mg^{2+}	Magnesium cation
$MgSO_4$	Magnesium sulfate
ML	Maximum -likelihood
Mn SO_4	Manganese Sulfatec
$MnSO_4$	Magnesium sulfate
MP	Maximum -Parsimony
Na_2CO_3	Sodium carbonate
NaCl	Sodium Chloride
$NaClO_3$	Sodium Chlorate

NaOH	Sodium Hydroxide
NCBI	National Center for Biotechnology Information
NH_4Cl	Chlonde Ammonium
Ni^{2+}	Nickel cation
$NiSO_4$	Nickel sulfate
Novo zymu35	Is a commercially available immobilized lipase produced by Novozymes it is based on immobilization via interfacial activation of lipase from *Candida* on tarectica.
Nox	Nitrogen Oxides
OMWs	Olive Mill Wastes
PCR	Polyemerase Chain Reaction
PDA	Potato Dextrose Agar
PGAP	Prokaryotic Genome Annotation pipeline
PMSF	Phenyl Methyl Sulfonyl Fluorid.
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SDSPAGE	Sodium Dodecyl Sulfate Polyacryl Amide Gel Electrophoresis.
SMF	Submerged Fermentation or Submerged liquid
SSF	Solid-State Fermentation
T	Time of the reaction (min)
TDS	Total Dissolved Olids
TFAT	Two Factors At a Time
TPOMW	Two-Phase Olive Mill wastes
TRIS-HCL	*TRIS Hydrochloride*
UK	United kingdom
V1	Volume of NaOH (ml).
V2	Volume of lipase used in the reaction
Virtis,model#6KBT ES-55,NY.USA)	Freeze dryer united states
Vivaflow 50	Laboratory Cross Flow Cassette
ZnCl	Zinc Chloride
$ZnSO_4$	Zinc Sulfate
$ZnSO_4$	Zinc sulfate

ABSTRACT

The current study aimed to the production and partial purification of a cold-active lipase by some fungi isolated from the olive oil processing wastes in Al-Gabal Al-Gharby, Libya. 31 fungal species from 12 genera were isolated with total CFUs of 29560. *Fusarium* was the most common genus encountering total CFUs of 9020 and comprising 30.51% of total fungi. *F. solani* was the most prevalent comprising 94% of total *Fusarium* and 28.7% of total fungi, 102 fungal isolates were tested for their lipolytic activity on lipase production agar medium at 10 and 20°C, The most active isolates were *Alternaria* (2 isolates), *Fusarium* (1), and *Penicillium* (1). Molecular identification of the most active four isolates was carried out by sequencing their (ITS). The four powerful fungal strains' production of cold-active lipase was maximized by optimizing some nutritional and environmental factors. *F. solani* AUMC 16063 was able to produce the maximum amount of lipase activity(46.66 U/mL/min) with specific activity(202.8U/mg) utilizing ammonium sulphate as a nitrogen source after 8 days of incubation at pH 3.0 and 15°C. However, at same condition after 6 days when yeast extract was employed as a nitrogen source, the generated cold-active lipase displayed the highest specific activity of (1550U/mg) and lipase activity (36.74U/ml/min). This is the first study in which the production, partial purification, maximized and characterization of a cold-active lipase enzyme by *Fusarium solani*.

Keyword: cold active enzymes, Lipase, *Fusarium solani*, lipolytic activity, specific activity.

1. INTRODUCTION

All living things have enzymes, which are biocatalysts. The specificity with which enzymes function, using little energy to catalyze a particular reaction, is what makes their biological activity distinctive. In all phases of metabolism and biological reactions, enzymes are essential. Because they can be utilized as catalysts in numerous biological reactions and hence have a wide range of applications, some enzymes are of particular interest(Ali *et al.* 2023).

The fermentation of biobased materials is how the majority of enzymes are created today and very certainly will be in the future (Louwrier 1998, Beisson *et al.* 2000). The majority of the biomass on earth is made up of lipids, and lipolytic enzymes are crucial for the metabolism of these water-insoluble substances. The breakdown of lipids within each individual organism's cells, which leads to their mobilization, as well as the transfer of lipids from one organism to another, are both facilitated by lipolytic enzymes (Beisson *et al.* 2000).

1.1. Microbial enzymes

Due to the wide range of catalytic activities that are available, the high yields that are feasible, the simplicity of genetic manipulation, the regular supply due to the absence of seasonal fluctuations, and the quick growth of microorganisms on inexpensive media, microbial enzymes are frequently more useful than enzymes derived from plants or animals. In addition to being safer and more convenient to produce, microbial enzymes are also more stable than their respective plant and animal enzymes (Liu and Kokare 2023).

1.2. Lipases (triacylglycerol hydrolases, E.C. 3.1.1.3)

Triacylglycerol acyl hydrolases (EC 3.1.1.3) are also known as lipases and are recognized as a group of potential industrial enzymes, responsible for catalyzing the hydrolysis or breaking down of insoluble fats and oils (triglycerides), and they can release monoglycerides, diglycerides, glycerol, and free fatty acids over an oil–water interface(Kumar *et al.* 2023).

The above groups are adaptable and enable a variety of bioconversion reactions in both unicellular and multicellular organisms, including hydrolysis, alcoholysis, acidolysis, aminolysis, esterification, and interesterification. Triacylglycerols (TAG) must be bioconverted both inside and between organisms, and lipases are crucial for this process(Geoffry and Achur 2018, Patel and Shah 2020, Ali *et al.* 2023).

Additionally, lipases are serine hydrolase familymembers that are carboxylic acid esterases which do not need a cofactor to catalyze events (Basheer *et al.* 2011). After proteases and carbohydrates, lipases are the largest family of digestive enzymes. In the realm of biotechnology, they represent the main class of biocatalysts (Carvajal Barriga and Portero Barahona 2019, Lima *et al.* 2019).

Furthermore, lipases have selective characteristics and catalyze the formation of esters, the hydrolysis of other esters, and their transesterification (Gupta *et al.* 2007). In the food, detergent, chemical, and pharmaceutical industries, lipases are becoming more and more preferred due to their capacity to carry out very specific chemical transformations (Grbavčić *et al.* 2007). The hydrolysis of triglycerides into glycerol and free fatty acids is catalyzed by lipases, a kind of hydrolase (Figure 1).

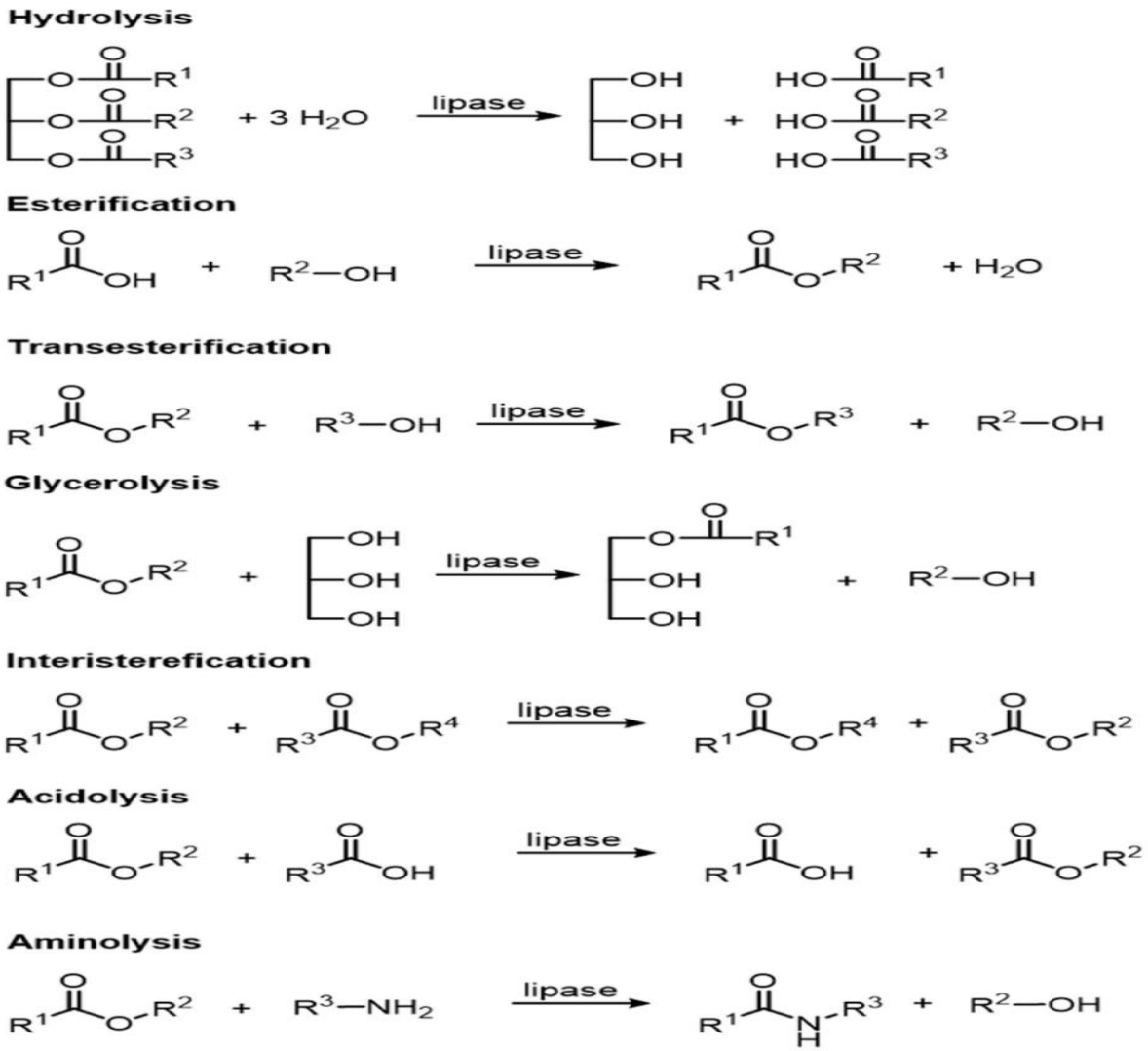

Figure 1. Reactions catalyzed by lipases(Salgado *et al.* 2022)

Lipases have great stability throughout a wide range of temperatures, pH levels, and even organic solvents, making them highly useful catalysts for reactions in both aqueous and non-aqueous conditions. Lipases have a hydrophobic lid that is essential for their interfacial activity (Khan *et al.* 2017, Mehta *et al.* 2017b, Tan *et al.* 2018, Bharathi and Rajalakshmi 2019).

Jamilu *et al.* (2022) mention the 1856 discovery of the lipase enzyme in pancreatic juice. Lipase was capable of hydrolyzing oil and fat droplets into soluble and digestible molecules. Due to their potential to significantly contribute to the expanding, multibillion-dollar bioindustry through both *in situ* lipid metabolism and *ex situ* different commercial uses, lipases have become one of the top biocatalysts/bio-accelerators (Joseph *et al.* 2008). Fungi that can create lipases can be found in a variety of environments, such as oil-contaminated soil, vegetable oil waste, dairy product manufacturing facilities, seeds, and expired food (Ko *et al.* 2005).

Furthermore, the significance of lipases for organic synthesis has grown because of the quick advancement of molecular biology procedures and the accessibility of more trustworthy high-throughput screening methods. Second-generation biocatalysts have so far been created by customizing a highly active enzyme for a specific application (Reetz 2001, Goddard and Reymond 2004). The most recent lipolytic bacterial strains are utilized (Vargas *et al.* 2004). The likelihood of industrial usage for lipases has increased as a result of numerous methods developed to get higher conversions for the particularly precise enzymes necessary for each application (Elemuo *et al.* 2019).

1.3. Lipase-producing microorganisms

Among the numerous types of microbes, fungi and yeast are recognized as possible sources of fungal lipase. Fungi produce extracellular lipolytic enzymes that are simple to extract and purify, which lowers production costs and makes them the preferred source over bacterial lipases. Fungal lipases are substrate-specific and stable under a wide range of chemical and physical conditions(Tan *et al.* 2003, Mehta *et al.* 2017b).

The most common fungal strains today produce commercial lipases in their culture medium, including; *Candida rugosa*, *Rhizopus oryzae*, *Mucor miehei*, *Rhizopus japonicus*, *Rhizopus arrhizus*, *Rhizopus delemar*, *Rhizopus niveus*, *Aspergillus niger*, and *Thermomyces lanuginosus*.

Commercial lipases derived from fungi are used in a variety of industrial fields, including; the production of detergents, food and dairy products, pharmaceuticals andmedicine, biodiesel,

oleochemicals, leather industry, also used in wastewater bioremediation, cosmetics and perfumeries, ester synthesis, paper manufacturing, and bioremediation(Chandra *et al.* 2020, Kumar *et al.* 2023). Due to their selectivity and advantages for future development, fungal lipases have enormous promise as biocatalysts for the creation of biomolecules (Mehta *et al.* 2017a, Geoffry and Achur 2018, Mahfoudhi *et al.* 2022). The following are the most significant advantages:

• They have high efficacy under mild reaction conditions.

• Easier to practice in the natural reaction mediumand products.

• Capable to decrease contamination from the environment.

• Accessibility of lipases from diverse fungal sources.

• Enhancement of catalytic power of lipases through genetic engineering.

1.4. Factors affecting production and activity of fungal lipases

The presence of various inducers, such as carbon sources, nitrogen sources, pH, and temperature, are the main parameters taken into account. These factors substantially influence the development and catalytic activity of lipases.

1.4.1. Carbon source

The induction of genes encoding the lipase enzyme in microorganisms is crucial for the generation of lipases from fungus. In order to stimulate the genes that code for the lipase enzyme in all types of microbial sources (microorganisms), carbon supplies are crucial. Carbon supplies assist in enhancing the fermentation process, which in turn improves cellular metabolism and boosts fungal lipase activity (Alabdalall *et al.* 2020).

Olive oil, palm oil, and other vegetable oils have all been employed as inducers for the synthesis of the lipase enzyme in microorganisms, along with other number offatty carbon sources. According to Sethi *et al.* (2013), *Aspergillus terreus* produced a sizable amount of lipase when mustard seed oil was utilized as a carbon source in the growth medium. Fatima *et al.* (2021)have noted that when a mixture of sugar cane bagasse and olive oil cake is utilized as a carbon source, fungal strains are able to produce more lipases. When compared to other carbon sources, olive oil cake has been demonstrated to be an effective inducer of the lipase enzyme.

Bindiya and Ramana (2013)used variety of lipid sources, including mustard oil, neem oil, coconut oil, olive oil, sunflower oil, palm oil, and cucumber oil, as well as numerous non-lipid sources of carbon, including sucrose, lactose, maltose, glucose, galactose, xylose, fructose, and mannitol, to test the effects of these sources on the production of lipase.

Due to the fact that carbohydrates serve as monovalent carbon sources for lipase production, *Aspergillus niger* produced a low yield of lipase when starch and sucrose were present, but a high yield of lipase when fructose was used as a carbon source (Alabdalall *et al.* 2020). In *Acinetobacter* sp., tween 80 has been utilized to help improve lipase recovery (Li *et al.* 2001, Mahfoudhi *et al.* 2022).

1.4.2. Nitrogen source

Nitrogen sources play a significant role in the creation of lipase enzymes because they are essential for microbial development and the stimulation of lipase production. Various microorganisms produce lipase using variety of organic and inorganic nitrogen sources, including; peptone, tryptone, sodium nitrate, ammonium salts, urea, and yeast extract (Oliveira *et al.* 2016, Bharathi and Rajalakshmi 2019, Fatima *et al.* 2021).

When urea was added to the growing media of *Rhizopus* sp., this increased lipolytic activity was seen (Rodriguez *et al.* 2006, Mehta *et al.* 2017a). To produce lipase from *Aspergillus* sp., a mixture of peptone and another nitrogen extract has been used. A combination of nitrogen sources was also found to affect the activity of the lipase enzyme. When glucose and peptone served as the sources of carbon and nitrogen in *Trichoderma harzianum*'s growth medium, lipase activity increased to its highest level; conversely, glucose and yeast extracts had the lowest levels of lipase activity (Bharathi and Rajalakshmi 2019, Colonia *et al.* 2019).

Furthermore, to study the effects of various nitrogen sources on the production of lipase in *Aspergillus sydowii*, Bindiya and Ramana (2013) employed nitrogen sources with a concentration of 1% w/v. Yeast extract, $NaNO_3$, tryptone, KNO_3, NH_2CONH_2, beef extract, and NH_4Cl were the nitrogen sources used in the study. When employing NH_4Cl, the greatest activity of lipase output was attained.

1.4.3. Temperature

The production of microbial lipase is significantly affected by temperature, which can also alter the physical characteristics of cell membranes and affect the release of the extra cellular enzymes. In the shake-flask method, an ideal temperature is essential and plays a significant role in the secretion of enzymes. At a temperature of 37°C, a greater biomass output of lipase synthesis was attained(Mehta *et al.* 2017a, Bharathi and Rajalakshmi 2019).

According to research, lipase is stimulated to produce more when the temperature is raised to 38°C (Bharathi and Rajalakshmi 2019, de Souza *et al.* 2019). Lower temperatures have been found to reduce the lipase enzyme's output, whereas higher temperatures have been found to have an impact on its activity. Mukhtar *et al.* (2016)conducted research on the impact of various incubation temperatures, spanning from 25°C to 55°C, on the *Aspergillus niger* lipase enzyme synthesis.

The temperature at which lipase produced the most was 30°C, followed by 35, 40, 25, 50, and 55°C. Mahmoud *et al.* (2015) looked at how *Aspergillus terreus* lipase production was affected by different incubating temperatures, specifically 10, 20, 30, and 45°C. The lipase activity peaked at 45°C (15 U/mL), and then reduced when the temperature dropped to 30°C (12 U/ml), 20°C (9.5 U/ml), and 10°C (3.0 U/ml).

The *Botrytis cinerea* strain, according to Comménil *et al.* (1995), developed a temperature-sensitive fungal lipase that was most active at 38°C and completely inactive at 60°C. Additionally, the *Botrytis cinerea* lipase can be stable at ambient temperature and displays 98% of its initial activity after 48 hours of incubation. At 50°C, considerable stabilities were seen for *Nomuraea rileyi* and *Rhizopus oryzae*, which both displayed their greatest enzymatic activity at 60°C (Supakdamrongkul *et al.* 2010, Saranya and Ramachandra 2020). Recently, a lipase from *Cladosporium tenuissimum* was isolated and purified; the lipase's highest activity was reached at 60°C (Saranya and Ramachandra 2020).

1.4.4. pH of the medium

Bacterial-origin lipases often have a pH that is either alkaline or neutral. According to recent studies, both bacteria and fungi produce lipase more readily at alkaline or slightly neutral pH conditions (Ramakrishnan *et al.* 2016, Bharathi and Rajalakshmi 2019). When the pH of their lipase-producing media was kept slightly neutral, Taskin *et al.* (2016)reported that *Rhodotorula glutinis* HL 25 showed fair lipase activity. Acidic pH, on the other hand, encourages the synthesis of fungal lipases. When the pH of the reaction process was maintained

at 4.0, Turati *et al.* (2019)observed that fungal lipase produced more and was more active. According to Mahmoud *et al.* (2015), *Aspergillus terreus* lipase output was affected by pH levels ranging from 2.0 to 12, at pH 8.0, the enzyme activity peaked.

1.4.5. Surfactants

Surfactants, which are ionic, long-chain organic compounds with hydrophobic and hydrophilic regions, can make the cell membrane more permeable, enabling the export of several other molecules through the membrane and promoting protein secretion. The internal charges of lipase can switch from cationic to anionic, and vice versa, when exposed to surfactants. As a result, lipases' physicochemical characteristics are altered (Essamri *et al.* 1998).

At every stage of enzyme production, purification, and characterization, surfactants are crucial gradients for emulsion preparations used in the lipase assay (Supakdamrongkul *et al.* 2010). According to Saranya and Ramachandra (2020), when surfactants like Triton X-100, Tween 20, SDS, and Tween 80 were employed in various quantities, multiple lipases showed divergence in their affinities to the substrate. The lipase generated by the fungus *Nomuraea rileyi* had increased activity when Tween 80 and SDS were present, but *Rhizopus oryzae* displayed decreased activity (Supakdamrongkul *et al.* 2010).

When added to the fermentation medium, surfactants like Tween 80, Tween 20, Triton X-100, and cetyltrimethylammonium bromide (CTAB) generally increase the activity of lipases, though the extent to which this is true may vary depending on the strain (Silva *et al.* 2005, Niaz *et al.* 2013, Das *et al.* 2017, Geoffry and Achur 2018). Therefore, choosing the right surfactant is crucial if you want strong lipase activity.

1.4.6. Moisture content

Particularly in solid-state fermentation processes, moisture content has a significant impact on the development of lipases and the proliferation of fungi (Singhania *et al.* 2009). Moisture has a variety of effects on the solid substrate's physical characteristics, including a reduction in substrate porosity, changes to the particle structure, the development of stickiness, a reduction in gas volume and aeration, and a restriction on oxygen diffusion in the substrate layer. The generation and activity of lipases are ultimately reduced by high moisture content, which also slows the filamentous growth of fungus. Hydrolytic activity and lipase synthesis are improved by having the ideal moisture content (Mukhtar *et al.* 2016, Oliveira *et al.* 2016).

1.4.7. Metal ions

Metal ions are an important component and have a variety of effects on the makeup and functionality of several enzymes, including lipases (Mahfoudhi *et al.* 2022). Different fungal lipases exhibited various responses to various metal ions. *Aspergillus japonicus* produces lipase, which is inhibited by Mn^{2+} and Hg^{2+} but stable in the presence of Ca^{2+}(Jayaprakash and Ebenezer 2012). Similar to this, Katiyar and Ali (2013) reported that *Candida rugosa* had enhanced lipase activity in the presence of Ca^{2+} ions. It's interesting to note that *Cladosporium tenuissimum* lipase activity is unaffected by Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}(Saranya and Ramachandra 2020). Metal ions such Cu^{2+}, Fe^{3+}, Hg^{2+}, Zn^{2+}, and Ag^{+} were found to suppress the lipase activity isolated from *Aspergillus oryzae*(Toida *et al.* 1995).

1.5. Applications of microbial lipases

Fungi lipases are an important class of enzymes with biotechnological significance because of their flexibility and ease of mass manufacture. Due to their flexible enzymatic properties and substrate selectivity, fungal lipases are very desirable for industrial applications. Industrial and specialty enzymes like lipases are increasingly being produced using recombinant DNA technology (Hasan *et al.* 2006, Salihu *et al.* 2012). When creating fine chemicals and food ingredients, lipase works as a biological catalyst. The synthesis of fine chemicals, manufacture of pharmaceuticals, creation of cosmetics, processing of fats and oils, food processing, leather, textile, formulation of detergent and degreasing agents, production of paper, and other operations all make substantial use of lipases (Lorenz *et al.* 2002). Figure 2 summarized the applications of lipase in some industries.

Figure 2. Industrial application of lipase (Akram *et al.* 2023)

1.5.1. Textile Industry

The oldest industries, which have been around for centuries, are the textile ones, and they have a big impact on the economies of many developing nations. One of humankind's basic needs is also met in part by the textile industry. Due to their extensive chemical consumption and consequent pollution, these sectors are dealing with extraordinary resource and environmental issues. Some substances cause cancer and have allergic reactions that are harmful to people's health(Rahman *et al.* 2020, Kumar *et al.* 2021).

Chemical waste from these companies, which includes heavy metals (lead and mercury), formaldehyde, chlorine, dyes, and detergents, causes complex environmental issues (Hooda 2020, Kumar *et al.* 2021). Since enzymes are dependable, stable, safe, and biodegradable, they are frequently used in place of hazardous chemicals to improve the environment and t0-he quality of manufactured goods. Textile industries are now switching from conventional methods to biological methods that involve the application of enzymes to replace these chemicals and solve these environmental problems (Kumar *et al.* 2021).

Fungal lipase is increasingly being used in the textile sector. Lipases help to remove size lubricants from fabrics, making the fabric more absorbent and improving levelness during dyeing. It also lessens the occurrence of streaks and cracks in denim abrasion sy stems. Lipase enzymes are present in commercial desizing solutions for denim and other cotton fibers (Salihu

et al. 2012). In the textile industry, polyester offers a variety of significant advantages, including as softness, high strength, washability, stain resistance, stretch, machine abrasion, and wrinkle resistance. Synthetic fibers are modified by enzymes to produce yarns, fabrics, rugs, and textiles. It deals with changing the characteristics of polyester fibers to make them more susceptible to post-modification treatments (Ali *et al.* 2023).

1.5.2. Detergent Industry

Hydrolytic fungal lipases are used as additives in commercial laundry and domestic detergents, which is their most lucrative commercial application (Verma *et al.* 2012, Ali *et al.* 2023). Lipase can decrease the negative effects of detergent products on the environment by allowing for lower wash temperatures while still conserving energy (Vakhlu and Kour 2006, Singh and Mukhopadhyay 2012, Bharathi and Rajalakshmi 2019). In 1994, Novo Nordisk introduced Lipolase, the first lipase used commercially. Lipolase was derived from the fungus *Thermomyces lanuginosus* and produced in *Aspergillus oryzae*. To eliminate stains from fabrics, fungal lipases are essential components of detergent mixtures. The business Novo Nordisk developed an enzyme that may remove fatty stains as early as 1988.

Although at a low concentration acceptable for commercial application, a certain strain of *Thermomyces* naturally generates this enzyme. After conventional approaches to boost yield were ineffective, the gene encoding for this lipase was cloned and inserted into *A. oryzae*. Manufacturers of detergent can use this fungus's lipase to increase washing effectiveness and save costs now that it has produced commercially feasible yields of the enzyme.Improved industrial lipase enzymes are more efficient at removing stains (Hasan *et al.* 2006). Only a few fungi, including *A. oryzae*, *Candida* sp., *R. oryzae*, and *Thermomyces lanuginosus*, have been demonstrated to manufacture lipases under controlled conditions that are suitable for detergent applications.

In contrast to soaps, which have washing qualities in water, detergents are sodium salts of long-chain benzene sulfonic acid or long-chain alkyl hydrogen sulfate. A detergent has both non-ionic (long-chain hydrocarbon) and anionic (sulfate or sulfonic) groups, which increase the detergent's water solubility. With the use of detergent, dirt and other pollutants can be combined and made more soluble in water without creating scum from the salt in hard water(Kumar *et al.* 2023).

Laundry and household detergents are becoming more and more common because of their anti-static qualities, water dispersibility, and capacity to soften cloth. Laundry and household detergents are currently available in a wide variety of brands, each with an own set of advantages. Increased detergent use pollutes the environment by releasing more detergent into the atmosphere. Additionally, these detergents raise the washing temperature (Verma *et al.* 2012).

Commercially significant is the use of hydrolytic fungal lipases as additives in the formulation of household and laundry detergents because these enzymes can reduce the environmental impact of detergent production by reducing the washing temperature of the intended detergent (Mehta *et al.* 2017a). In 1994, Novo Nordisk Company released the first lipase for commercial use. *Thermomyces lanuginosus* is a fungus that produces lipase, and *Aspergillus oryzae* expresses this enzyme.

1.5.3. Food Industry

Lipase is used in the food processing industry to degrade and alter biomaterials. The manufacturing of fat-clearing lipases on an industrial scale is common. The vast majority of commercially available lipases are employed in the processing of various foods, including meat, vegetables, fruit, smoked carp, milk products, baked goods, and beer. They are also utilized to improve the flavor of dairy products(Farahat *et al.* 1990, Pomeranz 2012).

Due to their speed, accuracy, economy of scale, and effectiveness, immobilized lipases make good triglycerol quantitative sensors. The immobilized lipase from *Rhizpous niveus*, newlase, has been used to add stearic acid to the sn-1 and sn-3 sites of the triglycerides in sunflower oil (Houde *et al.* 2004).

1.5.4. Pulp and Paper Industry

The pulp and paper industries are expanding quickly globally and are essential to the social, economic, and environmental progress of any nation. The need for paper will rise along with population growth and economic expansion. Wood, non-wood, and recycled waste paper are the three types of raw materials used in the production of paper (Abd El-Sayed *et al.* 2020).

In paper industries, some chemical compounds, such as hydrogen peroxidase, sodium carbonate, sodium hydroxide, sodium silicate, diethylenetriamine penta-acetic acid, and surfactants, are used in high amounts in different steps of conventional methods; these

chemicals are very toxic and become highly pollutant in the environment after releasing in wastewater. Several enzymes, such as lipase, amylase, esterase, cellulase, xylanase, hemicellulase, and pectinase, are used as a substitute for these chemicals to reduce toxic waste(Yakubu *et al.* 2019).

The presence of hydrophobic materials, such as triglycerides and waxes, in wood, the brassy reservoir of paper pulp and pitch, poses a highly challenging difficulty in the production of paper pulp. The completed product may have these triglycerides and waxes as holes, patches, or sticky deposits (Yakubu *et al.* 2019). Thus, during the production of paper, lipases can be used to separate the pitch from the pulpy material. About 90% of the pitch's glycerides are converted by lipases into less sticky and more hydrophilic diglycerides, monoglycerides, free fatty acids, and glycerol(Jaeger and Reetz 1998).

A solution to the pitch issue with wood was developed by Nippon Paper Industries in Japan utilizing *Candida rugosa* lipase, which can remove roughly 90% of the triglycerides from the pitch. Hata and Coworkers in Jujo's paper industry discovered in 1990 that lipases could lessen pitch issues by lowering the triglyceride level of pulverized wood pulp. *Candida cylindracea* produces lipase that, when added to the groundwood stock chest, can significantly lower the consumption of pitch and talc(de María *et al.* 2005, Mehta *et al.* 2017a).

1.5.5. Biodiesel production

The world's population, transportation, and industrialization are all growing steadily, which leads to an increase in the need for energy for both commercial and residential purposes (Santos *et al.* 2020). We rely mostly on fossil fuels (petroleum-derived diesel) to meet this rising demand for energy, but the reservoir of fossil fuels (petroleum-derived diesel) is finite and will eventually run out. The combustion of diesel releases a lot of CO_2, CO, and NOx gases into the air, which in turn causes pollution to rise and has a greenhouse gas effect on the planet (Quayson *et al.* 2020, Almeida *et al.* 2021).

As a result, we should try to look for alternate renewable energy sources that could meet our needs in the near future. With several advantages over fossil fuels, biodiesel has the potential to be a viable alternative resource for our energy needs (Santos *et al.* 2020). A blend of fatty acid alkyl esters make up biodiesel, which is non-toxic, biodegradable, and has high hexadecane and low sulfur content. In contrast to diesel made from petroleum, biodiesel emits less carbon dioxide (CO_2), carbon monoxide (CO), and nitrogen oxide (NOx) (Almeida *et al.*

2021). Additionally, by utilizing more biodiesel in this manner, we can lessen pollution and the effects of global warming.

There are many ways to make biodiesel, including dilution, pyrolysis, micro emulsion, and transesterification. The substrates used for transesterification to produce biodiesel include animal fats (like bovine tallow and lard), greases (like trap grease and float grease), leftover cooking oils, and vegetable oils (like rapeseed, cotton seed, palm, soybean, corn, jatropha, peanut, sunflower, and canola). Due to its low energy requirement and benign reaction conditions, the manufacture of biodiesel utilizing enzymes has grown in popularity recently(Vilar *et al.* 2018, Pérez *et al.* 2019, Carvalho *et al.* 2020).

There are two types of transesterification: catalytic and non-catalytic. A small chain alcohol (methanol, ethanol, or propanol), a catalyst (an acid, a base, and a catalyst), and either vegetable oil or animal fats are needed for the transesterification reaction (Almeida *et al.* 2021). In the transesterification process, enzymes, particularly lipases, serve as a catalyst for the generation of biodiesel. When exposed to benign physical conditions like pH, temperature, and pressure, lipases exhibit high catalytic activity, regioselectivity, and stereoselectivity. *Aspergillus niger*, *Rhizomucor miehei*, *Ralstonia* sp., *Candida rugosa*, *Candida antarctica*, *Rhizopus oryzae*, *Thermomyces lanuginosus*, and *Magnusiomyces capitatus* are just a few yeast and fungal lipases that have accelerated the generation of biodiesel(Fan *et al.* 2012).

In order to utilize it as a biocatalyst for the manufacture of enzyme-catalyzed biodiesel, Winayanuwattikun *et al.* (2011)immobilized *Candida rugosa* B lipase on seabeds EC-OD. Similar to this, Tian *et al.* (2021)revealed that *Rhizomucor miehei* lipase (RML), with just a single chain of α/β type protein, is heavily used in the production of biodiesel due to its high catalytic activity and tolerance against methanol.

1.5.6. Medical and Pharmaceutical Industry

The use of lipases as diagnostic tools is expanding quickly since their elevated levels are indicative of a number of disorders and also serve as key therapeutic targets or marker enzymes in the medical industry. Acute pancreatitis and pancreatic damage are two clinical conditions that can be identified by looking at the levels of lipase in the blood (Mahfoudhi *et al.* 2022). Lipase is a crucial enzyme for fat metabolism and lacking it can have harmful effects on one's health. According to Singh and Mukhopadhyay (2012), lipase activates tumor necrosis

factor, aids in the treatment of malignant tumors, and plays a critical role in the detection of heart conditions.

In the medical and pharmaceutical industries, lipases obtained from bacteria, fungi, yeast, and various protozoa have also been employed. In the presence of organic solvents, *Candida rugosa* produces a lipase that immobilizes on nylon supports and is utilized to synthesize lovastatin, a medication that is frequently used to treat blood cholesterol lowering (Yang *et al.* 1997, Mahfoudhi *et al.* 2022). Sharma and Kanwar (2014)reported that the fungus *Serratia marcescens* produces a lipase that can produce chiral 3-phenyl glycosidic acid by enantioselective hydrolysis. This intermediate compound is used to synthesize diltiazem hydrochloride, a medication used as a coronary vasodilator in many nations (Pérez *et al.* 2019). Similar to proteases, lipase can emulsify fats and may be utilized to treat problems of the digestive tract (Hasan *et al.* 2006). Another study found that lipases derived from yeast and fungi can be utilized as a therapeutic agent to treat gastrointestinal problems, dyspepsia, and cutaneous symptoms of digestive allergies(Mehta *et al.* 2017b).

1.5.7. Bioremediation of wastewater

In lipase biotechnology, the lipase-mediated bioremediation of wastewater is a unique, effective, and widely applied waste management method. When thin layers of fat are continuously scraped off the top of aerated tanks to preserve the oxygen supply, lipases can be used in activated sludge and other aerobic waste processes to break down the scraped-off, rich liquid (Cruz *et al.* 2020). Oil spills in coastal environments can be cleaned up using fungi, which could improve eco-restoration and aid in the enzymatic processing of oil in industrial settings(Gopinath *et al.* 1998).

Recently, fungi from the genera *Aspergillus*, *Cladosporium*, *Fusarium*, *Trichoderma*, *Mortierella*, *Penicillium*, and *Beauveria*, have been recognized as possible bioremediation agents in soil (Islam and Datta 2015). According to Singh and Mukhopadhyay (2012), *Candida rugosa*'s lipase used as an anaerobic digester. Several industrial operations, such as the treatment of sewage, the cleaning of holding tanks, septic tanks, grease traps, and PVC pipes, involve the use of lipases to break down particles efficiently and remove and avoid fat blockages or films in the waste system(Singh and Mukhopadhyay 2012).

In order to bioremediate contaminated soil and degrade polyvinyl alcohol films, *Aspergillus terreus* and *Aspergillus niger*, respectively, create lipases (Mahmoud *et al.* 2015, Mehta *et al.* 2017b). *Aspergillus uvarum* and *Aspergillus ibericus,* lipases can be used in

bioremediation procedures as well (Salgado *et al.* 2016). The solid assemblages of *Penicillium restrictum* and *Aspergillus niger*, both of which were isolated from oil-polluted soil and tested for lipase production, were found to treat synthetic mobile dairy effluent anaerobically with or without pre-hydrolysis by Leal *et al.* (2006).

According to research conducted by (Mauti *et al.* 2016), the lipase was discovered to biodegrade poly-aromatic hydrocarbons identified in soil contaminated by petroleum. *Aspergillus awamori* BTMFW032 was isolated from seawater and was capable of producing lipase and reducing 92% of the fat and oil content in mill effluent made up of waste oil (Basheer *et al.* 2011). Using a lipase isolated from *Geotrichum candidum*, olive mill effluent can be bioremediated and its decolorization can be managed (Kumar *et al.* 2020).

1.5.8. Cosmetics

Lipases have been used extensively in the personal care industry and the cosmetic industry for softening, cleansing, fragrance, and coloring. After the food and pharmaceutical industries, this industry has a sizable market value (Ansorge-Schumacher and Thum 2013, Mehta *et al.* 2017b). Due to their actions in the generation of surfactants and aromas, lipases have a significant potential for usage in cosmetics and perfumeries (Mehta *et al.* 2017b). Glycerol is esterified to create mono- and diglycerides, which are employed as surfactants in the fragrance and cosmetics industries. The production of rose oxide by the transesterification of 3,7-dimethyl-4,7 octadien-1-ol is catalyzed by lipases and is a crucial component of scent in the perfume industry(Izumi *et al.* 1997).

Moreover, Mouad *et al.* (2016) have reported that immobilized *Rhizomucor miehei* lipase is used as a biocatalyst in making personal care products such as skin creams and bath oils. In addition, *Candida antarctica* B yields a lipase, which synthesizes amphiphilic compounds that attain great attention in the cosmetic industry because they have a range of beneficial characteristics for the skin (Mouad *et al.* 2016). Unichem International (Spain) has produced isopropyl palmitate, isopropyl myristate, and 2-ethylhexyl palmitate for use as an emollient in personal care products such as skin and sun tan creams and bath oils, and the company also claims that the use of lipases as a substitute for conventional acid as a catalyst can improve the quality of the product with minimum downstream refining (Hasan *et al.* 2006).

In addition to being used to produce hair waves, lipases are also used for oral or topical anti-obesity treatment (Mehta *et al.* 2017b). As water-soluble retinol derivatives are created

through the catalytic reaction of immobilized lipase, vitamin A (retinol) and its derivatives have been employed extensively in pharmaceuticals and cosmetics, including skin care products(Mahfoudhi *et al.* 2022).

Propylene glycerol mono-fatty acid ester was created by Nippon Oil and Fats Co. Ltd. in the presence of lipase enzyme; this ester is employed as an emulsifier and pearling agent in the cosmetic and food sectors (Kim *et al.* 1997). In another study, *Candida antarctica* B lipase was covalently immobilized on hydrophobic and macroporous polyacrylate beads in a solvent-free media to produce n-butyl palmitate (a substantial valuable cosmetic component).

1.5.9. Biosensors

The quantitative determination of triglycerides, primarily fats and oils, in the food industry and clinical diagnosis are of paramount importance, and the biosensor, a lipid sensing device that is less expensive and more quickly operational than chemical methods of triglyceride determination, can carry out this function (Mehta *et al.* 2017b). According to Singh and Mukhopadhyay (2012), a biosensor device based on the enzyme-catalyzed disintegration of biodegradable polymer films has been developed. Three polymerase-enzyme systems were studied by (Sumner *et al.* 2001), including a dextran hydrogel that was broken down by the enzyme dextranase, a poly (ester amide) that was broken down by the protein degrading chymotrypsin, and a poly (tri-methylene) succinate that was broken down by a lipase enzyme.

The primary idea behind the lipase biosensor is to measure the amount of glycerol produced from triglycerides in an analytical sample using chemical, enzymatic, and colorimetric techniques (Hasan *et al.* 2006, Pérez *et al.* 2019). Additionally, glucose oxidase immobilized on pH oxygen electrodes and fungus and yeast lipases can be employed as lipid biosensors and for the measurement of triglycerides and blood cholesterol (Imamura *et al.* 1989). Fungal lipases are also employed in clinical diagnosis as biosensors to determine the concentration of lipids. One such lipase generated by *Candida rugosa* has been used as a DNA probe (Benjamin and Pandey 2001). For the purpose of detecting organophosphate insecticides, *Candida rugosa*'s lipase was immobilized on aluminosilicate.

1.5.10. Leather industry

The production of commodities including bags, shoes, clothing, belts, and purses by the leather industry contributes significantly to global employment, foreign exchange revenues,

and economic growth. However, the release of multiple harmful chemicals employed in various tanning process steps by these enterprises also causes environmental damage. The importance of leather and leather products in the global fashion business has led to the establishment of numerous leather industries in recent years. Raw animal skin and skins are transformed into leather during the manufacture of leather using a variety of mechanical and chemical procedures, including soaking, dehairing, bating, degreasing, and post-tanning(Dixit *et al.* 2015, Khambhaty 2020).

Different tanning processes use a variety of chemicals that are then discharged as waste, which significantly increases the chemical oxygen demand (COD), total dissolved solids (TDS), sulfates, chlorides, and heavy metals in the wastewater that is then released into water bodies like rivers, lakes, and the sea, greatly polluting them (Dayanandan *et al.* 2013, Dixit *et al.* 2015, Khambhaty 2020). Enzymes have been identified as the greatest practical option to utilize while tanning in order to limit the application of these hazardous chemicals, and these enzymes can aid in waste management(Khambhaty 2020).

Because collagen fibers in animal skin and hides contain both protein and fat, lipases are a crucial group of enzymes in the many steps of making leather. Before being tanned, these skins and hides must be partially or entirely removed. In particular, lipases disintegrate lipids without harming leather and demonstrate that the degreasing procedure has a smaller environmental impact (Choudhary *et al.* 2004, Dayanandan *et al.* 2013, Mehta *et al.* 2017b). As a result, of their high yield, quick fungi growth on less expensive media, and more dependable, simple, and secure production processes, fungal-origin lipases are also more effective than those originating from animals and plants.

Aspergillus tamarii MTCC5152 produced lipase at a high level (758±3.61 U/g) by solid-state fermentation. It also produces crude lipase (3%) that is used for tanning fleshing and has a 92% fat solubility rate (Dayanandan *et al.* 2013). Similar to this, Moujehed *et al.* (2022) demonstrated that the yeast *Yarrowia lipolytica* produces Lip2 lipase, by which sheepskins can be successfully degreased, which can successfully degrease even in 15 minutes at pH 8.0 and 30°C using only 6 mg/kg of raw skin.

1.5.11. Synthesis of biodegradable biopolymer and esters

Many researchers have turned their attention to biopolymers with high levels of diversity and complexity, such as polysaccharides, polyphenols, and polyesters since they may

be made from naturally available resources and are also biodegradable (Pérez *et al.* 2019). It is interesting to note that fungal lipases are used as biocatalysts in the synthesis of chemicals that degrade. The creation of 1-butyl oleate through the esterification of oleic acid and butanol lowers the viscosity of biodiesel when used in the winter (Hasan *et al.* 2006). Similar to this, the enzymatic transesterification of rapeseed oil fatty acids results in a mixture of 2-ethyl-1-hexyl esters that is produced in the proper proportion and serves as a solvent(Singh and Mukhopadhyay 2012).

Additionally, lipases play a role in the production of ester in transesterification processes in organic solvents in addition to acting as biocatalysts in the synthesis of biodegradable biopolymers. In the food business, short-chain fatty acid esters are employed as flavorings (Mehta *et al.* 2017b). According to (Janssen *et al.* 1999), the *Candida rugosa* lipase catalyzes the esterification process between fatty acids and sulcatol in toluene. These lipases were used as biocatalysts in a study to synthesize the aromatic esters geranyl butyrate, hexyl butyrate, and propyl butyrate, respectively, after being physically adsorbable on a Diaion HP-20 (hydrophobic and mesoporous support) (Dos Santos *et al.* 2021).

Under ideal conditions, the esterification process between lauryl alcohol and palmitic acid can produce more than 90% of lauryl palmitate thanks to the addition of *Candida antarctica* lipase (Novozym 435) as a catalyst. *Aspergillus ibericus* lipase can also be used as a catalyst in esterification processes and the synthesis of fragrance esters (Dos Santos *et al.* 2021). Mehta *et al.* (2017b)showed in another study that the esterification of ethanol and acetic acid results in ethyl acetate, whereas the esterification of ethanol and lactic acid results in ethyl lactate. Both of these processes are mediated by the lipase of *Aspergillus fumigatus*.

1.6. Cold-active lipases

The majority of the Earth's crust and atmosphere (85%) are subject to environments that are perpetually cold (5°C), which support psychrophilic microorganisms like algae, yeast, fungi, and bacteria (Kirchman *et al.* 2009). These microbes need a number of adaptation strategies to allow them to grow and carry out metabolic activity in such a cold environment if they are to survive. There has been a thorough review of these adaptation techniques used by psychrophiles as well as the underlying mechanisms(Gerday 2014, Collins and Margesin 2019).

Changes in membrane fluidity and the expression of cold-shock proteins, which are involved in transcription, translation, and posttranslational processes, result from adaptation to cold environments. Unsaturated fatty acids with more double bonds are present in the cell membrane of cold-adapted microbes. It is well known how these unsaturated fatty acids contribute to cold adaptation. The preservation of membrane fluidity during cold adaptation is thought to be a function of long-chain polyunsaturated fatty acids(Collins and Margesin 2019).

Lipases are present everywhere in nature and are active in a variety of temperatures. The temperature range of 0-30°C is favorable for the cold-active lipases' good activity (Cai *et al.* 2009). It is well known that many microbes, including bacteria, yeast, and fungi, are capable of producing cold active lipases(Abada 2008).

In extreme cold environments like Antarctica, deep sea environments, and refrigerated food samples, psychotropic and psychrophilic microorganisms are found to have a wide variety of cold-adapted lipases (Dieckelmann *et al.* 1998, Zeng *et al.* 2004, Dominguez de Maria *et al.* 2005). *Bacillus*, *Pseudomonas*, and *Burkholderia* are the three bacterial genera with the majority of significant lipase-producing organisms, while *Aspergillus*, *Penicillium*, *Rhizopus*, and *Candida* are the four fungal genera. Seven different genera, including *Zygosaccharomyces*, *Saccharomyces*, *Kluyveromyces*, *Pichia*, *Lachancea*, *Candida*, and *Torulaspora*, are home to some of the lipase-producing yeasts(Romo-Sánchez *et al.* 2010).

Two forms of cold active lipases that are stable at room temperature have been reported to be created by the mesophilic yeast *Geotrichum* sp., and cold active lipases active at 15℃ were produced by a bacterial strain called *Pseudomonas taiwanensis*(Khavitha and Shanthi 2013). *Pseudomonas gessardii*, a productive isolate that was isolated from oil-spattered soil from vegetable oil processing plants, has been shown to be able to manufacture extracellular lipase at mesophilic temperatures (Veerapagu *et al.* 2013). The DVL2 isolate, discovered in ordinary city trash, produced both extracellular and intracellular lipases that were active at mesophilic temperatures(Kumar *et al.* 2012).

1.7. Solid-state fermentation (SSF)

Submerged fermentation (SmF), also known as liquid state fermentation or submerged liquid fermentation, is the most popular method used to produce microbe-based products (such as microbial biomass, enzymes, cell metabolites, etc.) (Kurose *et al.* 2023). SmF could be expensive, leading to a process that is not economically viable, depending on the needs of the

microorganisms, the growth media, resources, energy inputs (such as significant amounts of water and expenditures for agitation and aeration), and equipment (Singhania *et al.* 2010). Additionally, SmF may be difficult to use for some enzymatic and metabolite releases since it is sensitive to a number of variables, vulnerable to contamination, and lacking in control over the process' physical and chemical variables(Singhania *et al.* 2010).

Several microbial biotechnology techniques use solid-state fermentation (SSF) as an alternative to SmF, in which bacteria and fungi are cultured on a wet, solid, non-soluble organic substrate in the lack or almost absence of free-flowing water (Lizardi-Jiménez and Hernández-Martínez 2017). SSF permits the bioconversion of organic agricultural and industrial wastes, accomplishing the circular economy goal (Ojo and de Smidt 2023), in addition to reduced energy usage and other practical advantages over SmF.

Industries with agricultural origins produce enormous amounts of residues every year. These leftovers could damage the environment and have an adverse effect on both human and animal health if they are not properly disposed of. Because the majority of agro-industrial wastes are untreated and underutilized, they are frequently dumped, burned, or unintentionally dumped into landfills. These untreated wastes produce more greenhouse gases, which exacerbate a number of climate change-related problems (Rodríguez Couto 2008, Sadh *et al.* 2018). The importance of adopting SSF to valorize various agro-industrial wastes to produce items with benefits for industry, agriculture, and human health was highlighted in a recent review(Yafetto 2022).

SSF is a three-phase heterogeneous process that incorporates solid, liquid, and gaseous phases to transform a starting substrate into products with added value. SSF has attracted a lot of attention in the last two decades for the development of industrial bioprocesses due to its economic and environmental sustainability while producing more products with a lower risk of contamination. The technical and financial viability of the process development is dependent on a number of elements that affect SSF. These criteria, just as with other bioprocesses (including SmF), involve choosing the optimal microbe/consortium and substrate as well as the best physical, chemical, and biological process parameters (such as pH, aeration, temperature, humidity, and solid material properties). Another factor that affects the viability of SSF production is product purification(Mattedi *et al.* 2023).

The heterogeneous nature of the substrate (a complicated gas-liquid-solid multiphase system) and heat accumulation throughout the fermentation are two of the primary SSF issues

to be solved in scale-up. SSF is frequently used to produce metabolites for biofuel, environmental applications (such as bioremediation), and the manufacture of metabolites (such as antibiotics, aromas, biosurfactants, enzymes, and organic acids)(Wang *et al.* 2010, Lizardi-Jiménez and Hernández-Martínez 2017).

1.8. Olive oil processing wastes (Fatura)

Table olives and olive oil are characteristic Mediterranean products with recognized economic and dietary benefits. Eating table olives and olive oil provides a range of health benefits, including a lower risk of heart disease and various types of cancer, according to numerous studies. These findings have prompted extensive research into the components of olive fruits and the kinds of olive fruit components that are accountable for the observed beneficial health effects(Tuck and Hayball 2002, Aruoma 2003, Fernández-Bolaños *et al.* 2006).

Oil content significantly affects lipase production. It was discovered that utilizing 2% of olive oil as an enhancer led to a maximum lipase activity of around 30.30 U/g starting dry weight after 24 hours of cultivation, as opposed to peptone's activity of 27.80 U/g initial dry weight(Rao *et al.* 1993).*Rhizopus oligosporous* GCBR-3 strain was shown to have a maximum activity of 30 2.1 U/g in earlier experiments, and employing *Rhizopus* strains also increased activity(Toshihiko *et al.* 1989, Manurukchinakorn and Fujio 1997).

AIM OF THE STUDY

The current study aimed to the production and partial purification of a cold-active lipase from some fungi isolated from the olive oil processing wastes (Fatura) in Al-Gabal Al-Gharby, Libya. This aim can be accomplished following the next objectives:

1. Isolation and identification of fungi associated with the olive oil processing wastes (Fatura) collected from different cities in Al-Gabal Al-Gharby, Libya.
2. Screening the cold-active lipolytic activity of the isolated fungi and selection of the highest cold-active lipase producers.
3. Optimization of some nutritional and environmental conditions for maximization of the cold-active lipase production.
4. Production of the cold-active lipase by the potent strain utilizing the Fatura waste as a substrate in solid-state fermentation, at the optimum production conditions.
5. Partial purification of the produced cold-active lipase using ammonium sulphate precipitation and dialysis.
6. Characterization of the produced cold-active lipase to determine the optimum pH and temperature, the best substrate for lipase activity, and effect of ions and some inhibitors on the lipase activity.
7. Testing the effectiveness of the enzyme produced.

2.PREVIOUS STUDIES

Cordova *et al.* (1998) produced the lipase using solid-state fermented *Rhizomucor pusillus* and *Rhizopus rhizopodiformis* fungal cultures. In a mixture of 50% of sugarcane bagasse and olive oil cake, the enzyme activity was 43.01 U/ml.

Bapiraju *et al.* (2005) optimized the lipase production by the mutant strain of *Rhizopus* sp., and the optimum activity was 29 U/mL.

Diaz *et al.* (2006) obtained extracellular lipases using *Rhizopus homothallicus* with lipase activities of 1,500 U/gds and 50 U/mL, by SSF and SmF, respectively.

Cho *et al.* (2007) showed that the maximum extracellular lipase production (68 U/ml) was achieved at pH 6.0 by *Penicillium chrysogenum*, the optimal activity for lipase was found in the range of temperature 30–37 °C.

De Azeredo *et al.* (2007) obtained lipase activities of 17 U/gds and 12 U/mL for SSF and SmF, respectively, by cultivation of *Penicillium restrictum*

Bysubmerged fermentation (SmF),Teng and Xu (2008)investigated the lipase production by *Rhizopus chinensis* and obtained, at the optimized experimental conditions, a maximum lipase activity of 14 U/mL.

In SSF, Kempka *et al.* (2008) investigated the lipase production by *Penicillium verrucosum* and the optimum activity was about 40 U/gram of dry substrate (gds).

Vargas *et al.* (2008)studied the lipase production by *Penicillium simplicissimum* and obtained an activity of 30 U/gds. Both *P. verrucosum* and *P. simplicissimum* were isolated from the babassu oil industry.

Yadav *et al.* (2011) produced, purified and characterized a cold active lipase by *Yarrowia lipolytica* NCIM 3639. The strain produced cell bound and extracellular lipase activity when grown on olive oil and Tween 80, respectively. The organism grew profusely at 20°C and at initial pH of 5.5, producing maximum extracellular lipase. The purified lipase has a molecular mass of 400 kDa having 20 subunits forming a multimeric native protein. Further the enzyme displayed an optimum pH of 5.0 and optimum temperature of 25°C.

Malilas *et al.* (2013) produced Lipase by *Penicillium camembertii* KCCM 11268 under solid state fermentation, and application to biodiesel production, the production process was optimized by using statistical experimental designs. The initial moisture content, cultivation time, inoculum size and concentration of basal medium were considered as the factors of optimum conditions for SSF. KCCM 11268 was cultivated in SSF using wheat bran as the substrate for lipase production. Under the optimized condition, lipase activity was reached

around 7.8 U/ml after eight days fermentation. To partially purify the lipase, ammonium sulfate (80% saturation) was added to the crude lipase solution and concentrated using a diafiltration (VIVAFLOW 50). The concentrated lipase solution was immobilized on silica gel by cross-linking method. Also, PCL was mixed with a commercial lipase solution from *Candida rugosa* (CRL), and this mixture was co-immobilized on silica gel. The immobilized and co-immobilized lipase activities were 1150.1 and 7924.8 U/g matrix, respectively. Palm oil and methanol were used as substrates and 1 mmol of methanol was added every 1.5 h and 2 times during biodiesel production. The reaction was carried out at temperatures of 30, 40, 50, 60 and 70^{o}C. The maximum biodiesel conversion by co-immobilized lipase was 99% after 5 h at 50^{o}C. Jadhav *et al.* (2013) produced a lipase from *Halomonas* sp. BRI 8. The highest lipase production was observed in the medium containing olive oil and peptone. The optimum pH and temperature for enzyme catalysis were 7.0 and 10°C respectively.

Maharana and Ray (2014) Their investigation aimed to study psychrotrophic micro-fungi of soil in Jammu city, India. The fungal isolates were identified by morpho-taxonomically and screened for their ability to grow at low temperatures. Most of the predominant isolates were species of *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp., and *Fusarium* sp. Isolated micro-fungi were characterized and screened in respective enzymatic agar medium for their degradation capability at 15°C. *Penicillium* sp., *Fusarium* sp., *Alternaria* sp. were found to be the maximum producer of cold active lipase, whereas *Aspergillus* sp., and *Microsporum* sp. showed maximum cellulase activity. The findings of this study indicate the possibility that the isolated strains produce novel extracellular enzymes that were active in cold temperature, which has immense application in many industries.

Tripathi *et al.* (2014) produced and purified a lipase from *Microbacterium* sp. The purified lipase exhibited maximal hydrolytic activity at a temperature of 50°C and a pH of 8.5 with highest specific activityof4.9 U/mg.

Bae *et al.* (2014)utilized *Pichia lynferdii* Y-7723 to produce a cold-active lipase. The enzyme produced the activity peak at pH 8.0 and 15°C, and pH 7.5 at 30°C. At 15°C, 1 mM of metal ion. Cu^{2+} and Co^{2+} showed slightly enhanced activity with 31 and 38%, respectively compared to the control.

In India, Lanka and Latha (2015) produced an extracellular cold-active lipase by mesophillic fungi, isolated from the soil samples of palm oil mill effluent dump sites, and was identified as *Emericella nidulans*. The enzyme was purified by ammonium sulfate fractionation followed by hydrophobic interaction chromatography using phenyl sepharose. The enzyme was 35 fold pure compared to crude with a specific activity of 1494.51 U/mg. SDS PAGE analysis revealed

that the protein is monomeric with a MW of ˜54 kDa and zymogram analysis showed that the purified protein was active. Characterization studies revealed that the temperature optimum was at 30°C and an optimum pH of 5. The Km and Vmax values were found to be 0.61 mM and 322.58 mM/min.mg, respectively. Sequencing of the purified protein by MALDI TOF-MS analysis followed by BLAST P analysis indicated that the protein is a putative secretary lipase from *E. nidulans*. Search of lipase engineering data base (LED) revealed that this protein belongs to a newly introduced super family of *Candida antarctica* lipase A like and to the homologous family of *Aspergillus* lipase like.

Yong *et al.* (2016) In this paper, extracellular lipase of *Botryococcus sudeticus* UTEX 2629 was isolated and studied. This is the first time lipase isolated from *B. sudeticus* is reported. The optimum lipase production condition was analyzed by incubating *B. sudeticus* in various types of oil (palm, corn, canola and olive oils) and under different agitation effects. The highest lipase production was found in the olive oil containing medium with the presence of agitation. The molecular mass of the purified lipase was estimated to be approximately 120 kDa. The kinetic properties of the purified lipase were determined and the values for Vmax and Km of the purified lipase were 33.00 mol min^{-1} mg^{-1} and 2.02 mM respectively. The lipase was active in high temperatures ranging from 40 to 70°C and in alkaline buffers from pH 10 to 11. It showed maximal activity at 50°C and pH 10. There was stable and enhanced activity after 12 h pre-incubation at 30°C. The lipase has highest specificity for the medium alkyl chain substrate. Finally, metal ions $FeSO_4$, $MnSO_4$, NaCl, ZnCl and $ZnSO_4$ and reagents SDS, EDTA, PMSF, ethanol and isopropanol were found to enhance enzyme activity.

Wongwatanapaiboon *et al.* (2016) Notice that *Fusarium solani* NAN103 lipase was successfully overexpressed in *Pichia pastoris* using inducible expression system and constitutive expression system under the control of alcohol oxidase 1 promoter (pAOX1) and glyceraldehyde-3-phosphate dehydrogenase promoter (pGAP), respectively. Lipase obtained using the constitutive promoter showed the highest activity of 18.8 U/mg in 3 days of cultivation time. Optimal lipase activity was observed at pH 7.0 and 35°C using pnitrophenyl laurate as the substrate. Lipase activity was enhanced by Mn_2C, Ba_2C, LiC, Ca_2C, Ni_2C, CHAPS and Triton X-100 but was inhibited by Hg_2C, AgC and SDS. The addition of 10% v/v of octanol, p-xylene, hexane and isopropanol increased lipase activity. Cultivation of lipase-expressing *P. pastoris* under pGAP in synthetic wastewater containing 1% w/v palm oil resulted in degradation of 87% of the oil within 72 h. *P. pastoris* expressing *F. solani* lipase from constitutive expression system has the potential to be used as an alternative microorganism for lipid degradation.

Sharma *et al.* (2017)producedlipase from *Bacillus methylotrophicus* PS3. The purified lipase presented maximal hydrolytic activity at a temperature of 55°C, and pH of7.0. Lipase activity was stimulated by Triton X-100 and SDS with Mg^{2+} and Ca^{2+} metals employa positive effect and outlast its stable in organic solvent i.e. methanol and ethanol.

Sahay and Chouhan (2018) produced lipases from psychrotrophic fungal isolates BPF4 and BPF6 identified as *Penicilium canesense* and *Pseudogymnoascus roseus* respectively were characterized for their compatibility towards laundry detergent. BPF4 and BPF6 lipases showed maximum activity at pH11 and 9 respectively and at 40°C. The residual activities at 20ºC and 4ºC of BPF4 lipase were 35% and 20% and of BPF6 lipase were 70% and 20ºC respectively. Both the enzymes were stable at 4ºC, 20ºC and 40ºC for 2h losing at the most 20% of activities. Both the enzymes were metalloenzymes with activity enhancement by nearly threefold by Ca^{2+}. Contrary to BPF6 lipase, BPF4 enzyme was not stimulated by EDTA nor inhibited, rather stimulated by SDS and Triton X-100 by 125% and 330% respectively. Both the lipases showed minor to moderate inhibition by $NaClO_3$ and H_2O_2, and exhibited nearly 90% residual activity after 1h of incubation in selected detergent brands thus indicating potential for their inclusion in detergent formulation thereby facilitating cold-washing as a step towards mitigation of climate change.

In Biotechnology Reports Journal study for Li *et al.* (2018) an extracellular lipase gene from *Yarrowia lipolytica* (LIPY8) was cloned and expressed by baculovirus expression system. The recombinant lipase (LipY8p) was purified using chromatographic techniques, resulting in a purification factor of 25.7-fold with a specific activity of 1102.9U/mg toward olive oil. The apparent molecular mass of purified LipY8p was 40 kDa. The enzyme was most active at pH 7.5 and 17ºC. It exhibited maximum activity toward medium chain (C10) esters. And they noticed that the presence of transition metals such as Zn^{2+}, Cu^{2+}, and Ni^{2+} strongly inhibited the enzyme activity, which was enhanced by EDTA.

In journal of Microbial technology, scientific investigation forHassan *et al.* (2022)produced a cold-active lipase from marine *Bacillus cereus* HSS, isolated from the Mediterranean Sea, Alexandria, Egypt. The enzyme was purified and characterized. The total purification depending on lipase activity was 438.9 fold purification recording 632 U/mg protein. The molecular weight of the purified lipase was estimated to be 65 kDa using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The optimum pH and temperature were pH 6 and 10°C, respectively.

Esakkiraj *et al.* (2022) expressed the lipase gene from *Psychrobacter celer* PU3 into pET-28a(+) expression vector and over expressed in *E. coli* BL21 (DE3) pLysS cells. The purified *Psychrobacter celer* lipase (PCL) was optimally active at 30°C and pH 8.0.

Mendes *et al.* (2022) produced a lipase by using *Fusarium solani*. After optimization using DCCR, maximum lipolytic activities of 24.84 U/mL were obtained with the use of 10 g/L tryptone, 3.50 g/L calcium chloride and 0.50 g/L magnesium sulfate, 1 g/L potassiumphosphate and 1% soybean oil. The biochemical characterization of lipase showed that the enzyme has a better performance at pH 7 at a temperature of 40°C.

Khan *et al.* (2023) used the *Bacillus pumilus* strain WSS5 to produce a lipase enzyme that has a specific activity of 17.3 U/mg. The purified lipase exhibited an optimum pH of 8 and temperature at 37°C. The oil-destaining activity of that enzyme was least for treatment with only plain water, suggesting its inefficiency in removing the stain. Treatment with water along with detergent alone did not promote the efficient removal of oil stains. However, complete removal of stain was observed when a combination of water with detergent along the purified enzyme was used.

3. MATERIALS AND METHODS

3.1. Collection of Fatura samples

In Libya during 2022, seven composite samples of the waste products from the manufacturing of olive oil (Fatura) were gathered from Zintan, Rayaina, Jadu, Yafran, Rujban, Al-Asabaa, and Gharyan. In order to isolate fungus, samples were put in sterile plastic bags and brought right to the mycological lab.

3.2. Isolation and preservation media

Czapek's Dox agar was used as an isolation medium. The medium contained (g/L): Sucrose, 30; sodium nitrate, 2; di-potassium hydrogen phosphate, 1; potassium chloride, 0.5; magnesium sulphate, 0.5; zinc sulphate, 0.01; copper sulphate, 0.005; agar, 20. Rose Bengal at 0.05 g/L and chloramphenicol at 250 mg/L (Smith and Dawson 1944, Al-Doory 1980) were added to suppress the growth of bacteria and to restrict the fungal colonies which facilitate the isolation of slow-growing fungi.

Potato dextrose agar (PDA)was used for preservation of the isolated fungi. The medium contained (g/L): Infusion from 200 g potato, glucose, 20, and agar, 20. The pH was adjusted to 7.0.

3.3. Determination of moisture content (MC)

Used method of Van Reeuwijk (2002): Twenty grams of Fatura samples were separately weighed and dried in an oven at 105^{o}C over-night, then cooled in a desiccator for at least 30 minutes and reweighed and the moisture content was calculated as follow:

$$\textbf{\% Moisturecontent} = \frac{\text{wt.of wet sample} - \text{wt.of dry sample}}{\text{wt.of wet sample}} \text{x } 100$$

3.4. Isolation of fungi from Fatura samples

The dilution plate technique (Warcup 1950)was used for isolation of fungi from the collected Fatura samples. In this method, 20 grams of each sample were individually placed in Erlenmeyer conical flask contains 80ml sterile distilled water and was shacked for 60 min at 150 rpm to make a spore suspension of the sample. 1 mL of the spore suspension was separately

transferred to sterile Petri plates that containing the isolation medium. The plates were then incubated at 25°C for 10 days. Five plates were used for each sample. The developed colonies were purified on Cz-agar medium to obtain pure cultures of fungi. Colony forming units (CFUs) of each isolate fungal species was calculated according to the following Equations (1-2):

$$\textbf{CFUs} = \frac{\text{Total count of each fungal species x Dilution factor}}{\text{Number of plates for the sample}} \textbf{(1)}$$

$$\textbf{CFUs} = \frac{\text{Total count of each fungal species x 5}}{5} \textbf{(2)}$$

3.5. Phenotypic identification of fungi

The morphological identification of the isolated fungal genera and species was based on macroscopic and microscopic features following the keys and descriptions of: Ellis (1976) for Dematiaceous Hyphomycetes, Pitt (1979) for *Penicillium* and its teleomorphs, Raper and Fennell (1965) for *Aspergillus*species, Booth (1971) and Leslie and Summerell (2006) for *Fusarium* species, Moubasher (1993) and Domsch *et al.* (2007) for fungi in general.

3.6. Preservation of the isolated fungi

All the isolated fungal strains were preserved in the culture collection of the Assiut University Mycological Centre (AUMC) using three different methods of preservation, namely on PDA slants at 4°C, in 15% glycerol water (Glycerol, 15mL and distilled water, 85mL) at -80°C, and as lyophilized ampoules.

3.6.1. Preservation of fungi on agar slants at 4°C

Test tubes with a 15 mL capacity were used to prepare and distribute Czapek's agar (CzA) and potato dextrose agar (PDA). The fungal strains that needed to be preserved were inoculated in the agar slants and sterilized at 121°C for 20 minutes before being incubated at 25°C for 7 days. The developed fungal slants were then kept in a frigid at 4°C for preservation (Smith and Onions 1994, Mukunda *et al.* 2012).

3.6.2. Preservation of fungi in glycerol water at -80°C

Two discs containing cultures of the fungal strains that needed to be preserved at a 7-day old were submerged in sterile 15% glycerol water solution and placed in sterile Eppendorf plastic tubes. After that, the Eppendorf tubes were kept at -80°C in an ultra-deep freezer (Ellis 1979, Smith and Onions 1994).

3.6.3. Preservation of fungi as lyophilized samples

In 10% skimmed milk and a 5% inositol solution, spore suspensions of the various fungal strains were created. Separate lyophilization glass tubes measuring 12 cm in length and 6 mm in diameter were used to distribute the fungi's spore suspensions that needed to be preserved. The tubes were then kept at -80°C for five hours in an ultra-deep freezer. The tubes were then moved to the freeze dryer, where they were dried at a very low temperature (-60°C) and atmospheric pressure of 1×10^{-5}. Using a gas/oxygen torch, the tubes containing dried fungal cultures were sealed after 36 hours (Smith and Onion 1994, Espinal-Ingroff *et al.* 2004).

3.7. Preliminary screening of lipolytic activity

The medium described by Ullman and Blasins (1974) was used for screening the lipolytic activity of the isolated fungi. The medium composed of (g/L): Peptone, 10; $MgSO_4$ $.7H_2O$, 0.2; $CaCI_2.2H_2O$, 0.2; Tween 80, 10 ml; and agar, 20. 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. The medium was dispensed aseptically in 15-cm test tubes (12 ml/tube). Test tubes were inoculated on the surface of agar by 50 µL of spore suspension obtained from 7-day-old cultures of the tested fungi. The tubes were then incubated at 10 and 20ºC for 14 days. The lipolytic ability was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. The depth of each visible precipitate (in mm) was measured. The most active fungal strains were selected for the optimization of lipase production conditions.

3.8. Optimization of Cold-Active Lipase Production Conditions

3.8.1. Optimization of pH and Temperature

By changing the parameters using the two factors at a time (TFAT) strategy (Moharram *et al.* 2022) for pH (3-10) each at 10, 15, and 20°C,it was possible to maximize the synthesis of cold-active lipase by *Alternaria alstroemeriae* AUMC 16060, *Penicillium crustosum*

AUMC 16061, *Alternaria angustiovoidea* AUMC 16062, and *F. solani* AUMC 16063.A 50 mL of the fermentation medium (Sucrose-free Czapek's broth) was added to each 250-mL Erlenmeyer flask used for the tests, along with 1.0% tween 80 as the only carbon source. Separate flasks were inoculated with 1.0% (v/v) of spore suspension taken from a 7-day-old cultures of the tested fungi. The flasks were then incubated for 10 days.

3.8.2. Optimization of Nitrogen Source and Fermentation Time

Two factors at a time (TFAT) strategy (Moharram *et al.* 2022), nitrogen supply (peptone, yeast extract, beef extract, sodium nitrate, ammonium sulfate, and ammonium chloride; each at 0.2%) each at a fermentation duration of 1–10 days, were varied for the maximization of the cold-active lipase by *F. solani* AUMC 16063. A 50 mL of the fermentation medium (Sucrose-free Czapek's broth) was added to each 250-mL Erlenmeyer flask used for the tests, along with 1.0% tween 80 as the only carbon source. Separate flasks were inoculated with 1.0% (v/v) of spore suspension taken from a culture of *F. solani* AUMC 16063 that had been growing for seven days. The flasks were then incubated under various operating conditions.

3.8.3. Extraction and assay of the Cold-Active Lipase

After fermentation duration, the cell-free supernatant was obtained through 10,000 rpm centrifugation at 4°C for 10 min, and used as the source of the cold-active lipase(Mayordomo *et al.* 2000). For quantitative determination of the lipolytic activity, 3 mL of 1.0% tween 80 was combined with 2.5 mL of deionized water and 1 mL of buffer solution respective to the desired pH tested. The mixture was maintained at 5, 10, 15, and 20°C for overnight. Afterwards, 1.0 mL of the supernatant was added to the mixture to start the reaction for 60 min. The reaction was then terminated by introducing 3 mL of 96% ethyl alcohol, and the mixture was titrated against 0.05M NaOH solution in the presence of 4 drops of phenolphthalein solution (0.1 g dissolved in 50 mL of 1:1 ethanol: distilled water), until the appearance of the pink color. The lipase activity was calculated according to Equation (3):

$$\textbf{Lipase activity} = \frac{\mathbf{V1 \times M \times 1000 \times DF}}{\mathbf{V2 \times T}} \textbf{U/mL/min} \qquad \textbf{(3)}$$

Where: V1 = Volume of NaOH (mL); 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).

3.9. Total Protein Estimation(Lowry *et al.* 1951)

3.9.1. Preparation of Lowry reagent

Solution A (500 mL)2.86 g NaOH and 14.3 g Sodium carbonate (Na_2CO_3) were dissolved in 500 mL distilled water.

Solution B (100 mL)1.4232 g of copper sulphate ($CuSO_4$). $5H_2O$ was dissolved in 100 mL distilled water.

Solution C (100 mL)2.853 g disodium tartarate was dissolved in 100 mL distilled water.

For preparation of Lowry reagent, 100 mL of solution A was mixed with 1 mL of solution B and 1 mL of solution C.

3.9.2. Preparation of Folin reagent

5 mL of 2N Folin and Ciocalteu's phenol reagent + 6 mL distilled water

The protein content was estimated according the Lowry *et al.* (1951) method. A 0.5 mL of the sample was mixed with 0.7mL of Lowry reagent and the mixture was then incubated in the dark for 20 min. At the last 5 minutes, the Folin reagent was prepared, and then 0.1mL of the Folin reagent was added to the mixture, and incubated for 30 min. After that, the absorbance was measured at 750nm (T80+ Spectrophotometer, UK). The protein content was then calculated using the standard curve of Bovine Serum Albumin (BSA) (Figure 3).

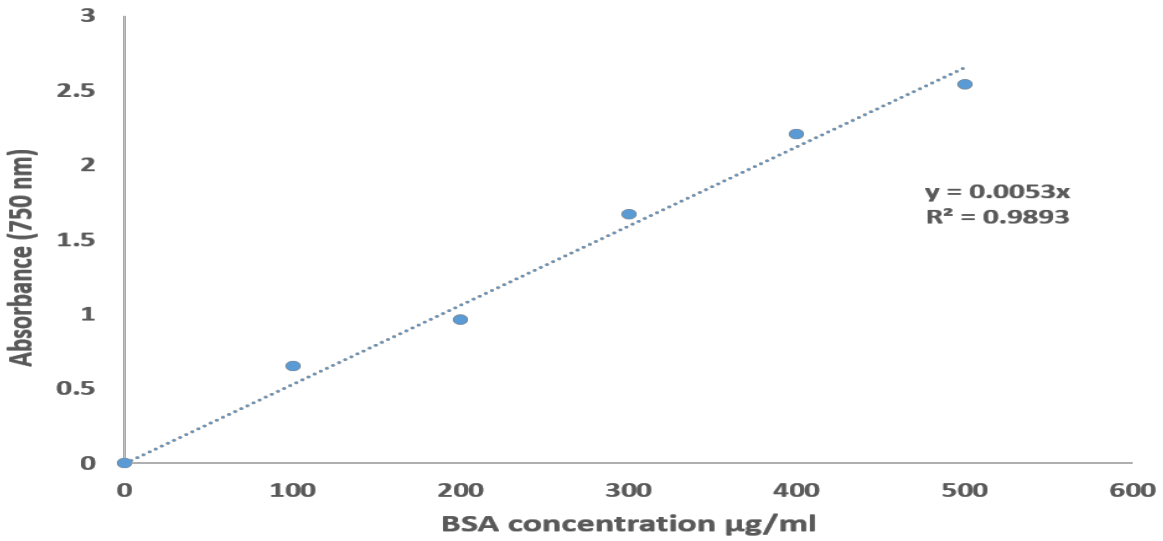

Figure 3. Standard curve of protein

3.10. Molecular identification of the potent fungi

3.10.1. DNA extraction

A small amount of 7-day-old fungal cultures grown on PDA was separately scraped and suspended in 200 µl of sterile distilled water in 2ml sterile vials and boiled at 100℃ for 15 minutes (Deak *et al.* 2000). A 800 µl CTAB buffer composed of 3 % CTAB, 1.4 M NaCl, 0.2 % Mercaptoethanol, 20 mM EDTA, 100 mM TRIS-HCl pH 8.0 and 1 % PVP-40, were added to each tube. After incubation at 65°C for 30 min, 800 µl of CI Mix with the composition of 24 ml chloroform and 1 ml isoamyl alcohol, were gently added and mixed with the tube contents. A clear supernatant was obtained by centrifugation at 10000 xg for 10 min. For DNA precipitation 2/3 volume of isopropanol (precooled at -20°C) was added and mixed gently. The samples were incubated at 4°C overnight, thereafter centrifugation at 13000 xg for 10 min. The supernatant was discarded, and the pellet was pooled and washed with 200 µl washing buffer composed of 76 % ethanol and 10 mM ammonium acetate. The washing buffer was carefully decanted, and the pellet was suspended in 200 µl TE buffer supplemented with 10 mg/ml RNase. After incubation at 37 °C for 30 min, 100 µl of 7.5 M ammonium acetate and 750 µl ethanol were added and mixed gently. Samples were centrifuged at 13000 xg for 10 min at room temperature. The supernatant was completely discarded, and the pellet was suspended in 100 µl sterile distilled water.

3.10.2. PCR for rDNA and sequencing using ITS1 and ITS4 primers

The universal primers ITS1 and ITS4 (White *et al.* 1990)were used for DNA amplification. In the PCR tubes 1µl of DNA template, 1µl 2.5mM dNTP mix, 0.2 unit of Taq polymerase, 5µl of 10x complete buffer and 40µl of sterile ddH_2O, 10pmol of ITS1 (5´ TCC GTA GGT GAA CCT TGC GG 3´) and ITS4 (5´ TCC TCC GCT TAT TGA TAT GC 3´) were added. Then the PCR amplification was carried out using the following sequence: one round of amplification consisting of denaturation at 95°C for 15 min followed by 30 cycles of denaturation at 95°C for 20 sec, annealing at 50°C for 40 sec and extension at 72°C for 1 min, with a final extension step of 72°C for 5 min. The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. The purified PCR products were confirmed on 1% agarose gel by electrophoresis using size marker. The bands were eluted and sequenced in the forward and reverse directions.

3.10.3. Alignment and phylogenetic analysis

Contiguous sequences of the fungal species in this study were uploaded to GenBank and accession numbers were given. Sequences of the nearest species were downloaded from GenBank including sequences of the available type specimens. All sequences in this analysis were aligned together using MAFFT(Katoh and Standley 2013)with the default options. Alignment gaps and parsimony uninformative characters were optimized by BMGE (Criscuolo and Gribaldo 2010). Maximum-likelihood (ML) and Maximum parsimony (MP) phylogenetic analyses were performed using MEGA X version 10.2.6 (Kumar *et al.* 2018). The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Felsenstein 1985). The best optimal model of nucleotide substitution for the ML analyses was determined using Akaike information criterion (AIC) as implemented in Modeltest 3.7 (Posada and Crandall 1998). The phylogenetic tree was drawn and visualized using MEGA X (Kumar *et al.* 2018). The resulting tree was edited using Microsoft Power Point (2016) and saved as TIF file(Al-Bedak et al. 2020).

3.11. Production of cold-active lipase from Fatura

According the method described by (Joseph*et al.* 2011)**,***Fusarium solani* AUMC 16063 was utilized to generate a cold-active lipase employing the Fatura waste as substrate under ideal production circumstances, which included an 8-day incubation period at pH 3 and 15°C with ammonium sulphate as a nitrogen supply. A 300g of Fatura were added to 3 Fernbach fermentation flasks (100g each), and each flask was moistened with 50mL of Czapek's broth, which was free of sucrose and enriched with 1g/L pectin derived from citrus peel. A 5 mL spore suspension from a 7-day-old culture of *F. solani* AUMC 16063 was used to individually inoculate each flask after sterilization. The flasks were then kept in a static environment for 8 days at 15°C. Following the incubation period, the contents of the flasks were collected using 1500 mL of citrate buffer (pH 3.0). After centrifugation (10,000 rpm at 4°C for 20 min), the cell-free supernatant was used for the enzyme precipitation.

3.11.1. Ammonium Sulfate Precipitation and Dialysis

Total protein was precipitated at 4°C with a 70% saturated ammonium sulphate(Al-Bedak *et al.* 2023). The precipitated protein was separated and lyophilized using a freeze dryer (VirTis, model #6KBTES-55, NY, USA). To remove salts and other small molecules,

lyophilized protein was dissolved in citrate buffer (pH 4.0), dialyzed twice for two hours at room temperature (cutoffs: 12-14 kD), and then cooled overnight at 4°C. Later, enzyme characterization tests were conducted using the partially purified cold-active lipase from the dialyzed protein.

3.12. Impact of pH, Temperature and Some Ions and Inhibitors on the Activity of the Cold-active Lipase

On pure lipase activity, the effects of pH (3.0–11.0) at 5–20°C have been examined(Ji *et al.* 2015). The reaction was started by adding 0.01 g of enzyme powder dissolved in 1.0 mL buffer solution of the desired pH values (3.0-11.0), along with 3.0mL of 1.0% tween 80, 2.5mL of de-ionized water, and 1.0mL of buffer solution. After 60 min, the reaction was terminated by introducing 3.0mL ethyl alcohol (96%), and the flask contents were titrated against 0.05 M NaOH. Additionally, ions such as Na^{+}, K^{+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} were tested by adding them to a solution at a concentration of 5 mM/mL as NaCl, KCl, $CaCl_2$, $CoCl_2$, $NiSO_4$, $CuSO_4$, $FeSO_4$, $MgSO_4$, $MnSO_4$, and $ZnSO_4$(Al-Bedak *et al.* 2023). An enzyme inhibitor was also tested using sodium dodecyl sulphate (SDS) and ethylenediaminetetraacetic acid (EDTA), both of which had a 5 mM/mL concentration. Under the standard reaction conditions of pH 4.0 and 10°C, the cold-active lipase's activity without the presence of metal ions, EDTA, or SDS was assessed to determine the residual activity.

3.13. Effect of Carbon Source on the Lipase Activity

The activity of the pure cold-active lipase generated in this investigation was assessed using 1.0% (w/v) of each of tween 20, tween 40, tween 80, maize oil, olive oil, sesame oil, and sunflower oil(Dalmau *et al.* 2000). A 0.01 g lipase powder was dissolved in 1.0mL citrate buffer (pH 4.0) and added to the reaction mixture, which included 3.0mL of 1% substrate, 2.5 mL deionized water, and 1mL citrate buffer (pH 4.0), for 60 minutes at 10°C. After stopping the reaction with 3.0mL of 96% ethyl alcohol, the flask content was titrated against a 0.05 M NaOH solution. The lipase activity was determined as previously stated.

3.14. Application of the Produced Lipase in Removal of Oily Waste from Clothes

Following the method outlined by Das *et al.* (2016), white cotton fabric, measuring 5cm × 5cm and free of dirt, was soaked for 15 minutes in corn, sesame, sunflower, and olive oils containing 0.1% Congo red dye. The cloth was then dried for 5 minutes at 80°C in a hot air

oven. The dried pieces were placed one at a time, individually, in Erlenmeyer conical flasks containing 50 U/mL of the produced lipase and shaken at 150 rpm for 60 minutes. The examined area was left in the open air to dry after being incubated for two minutes and then rinsed with tap water without scrubbing. For the lipase-free control, the same protocol was used.

3.15. Statistical Analysis

The mean and standard deviation (SD) of the tentative study performed in triplicate were used to express all data. Analysis of the statistical significance was conducted according to Stahle and Wold (1989). It was deemed significant at $p<0.05$.

`

4. RESULTS

4.1. Fungi recovered from Fatura

In this study, seven composite samples of olive oil processing wastes (Fatura) were obtained from Zintan, Rayaina, Jadu, Yafran, Rujban, Al-Asabaa, and Gharyan. 31 fungal species from 12 genera were isolated from these samples with total CFUs of 29560.*Fusarium* was the most common genus encountering total CFUs of 9020 and comprising 30.50% of total fungi. It was represented by two species, *F. oxysporum* and *F. solani*. *F. solani* was the most prevalent comprising 94% of total *Fusarium* and 28.7% of total fungi, while *F. oxysporum* encountered approximately 6.0% of total *Fusarium* and 1.8% of total fungi.

Fusarium was followed by *Aspergillus*, which made up 25.44% of all fungi and was represented by eight species. It appeared in all samples, which had a total CFU count of 7520. With 29.25% of all *Aspergillus* and 7.4% of all fungi, *Aspergillus aureolatus* was the most common species (Table 1).

After *Fusarium* and *Aspergillus*, *Penicillium* was ranked third. Nine different species were present. A total of 100% of samples were found to have CFUs of 5140 and 17.4% of all fungi. *Penicillium aurantiogriseum* was the species that was found the most frequently across 6 of the 7 samples. 5.8% of all fungi and 33.85% of all *Penicillium* were present in it.

The fourth most prevalent genus, which made up 14.14% of all fungi, was *Mucor*, which was represented by two known and one undetermined species. 4180 CFUs were discovered. The most common species, accounting for 88% of all *Mucor* and 11.9% of all fungi, was *M. hiemalis*. Below is a description of the study locations and isolated fungi:

4.1.1. Fungi isolated from Zintan

Eleven species related to seven genera comprising CFUs of 5700 were identified from Fatura collected from Zintan city at 25°C. Species richness was pronounced in genus *Aspergillus*, where it included 3 species (*A. aureolatus*, *A. niger* and *A. ustus*). It encountered 26% (1500 CFUs) of total counts of all fungi isolated. From these species, *A. niger* yielded the highest number of propagules (580 CFUs) followed by *A. ustus* (520) and *A. aureolatus* (400).

Penicillium and *Alternaria* (2 species each) were the runner of *Aspergillus*. *Penicillium* (11.6 % of total fungi) was represented by *P. roquefortii* (10.5%) and *P. citrinum* (1%). *Alternaria* constituting 5.6% (320 CFUs) of total fungi. *A. alstroemeriae* and *A. angustiovoidea* (200 and 120 CFUs) respectively. On the other hand, the genus *Fusarium* was represented by

one species only (*F. solani*) comprising the highest number of propagules (35.4% of total fungi). The remaining genera, *Microdochium*, *Mucor* and *Phialophora* were represented by one species each.

4.1.2. Fungi isolated from Rayaina

Five genera represented by thirteen species were recovered from this sample. Species richness was pronounced in both *Aspergillus* and *Penicillium*, they were represented each by 4 species. The genus *Aspergillus* showed the highest CFUs (2020 colony out of 4260). The most common species was recovered in high CFUs was *A. fumigatus*. *Penicillium* follows *Aspergillus* in the number of colonies (780 out of 4260 colony). It was represented by *P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum* and *P. citrinum* (constituting 6.1 of total count of *Penicillium*).The results also showed that genus *Mucor* was represented by 3 species (*M. hiemalis*, *M. racemosus* and *Mucor* sp.) and harbored 12.2% of total count of fungi in Rayaina sample. Whereas *Fusarium* (*F. solani*) and *Stachybotrys* (*S. chartarum*) were recovered with one species each as shown.

4.1.3. Fungi isolated from Jadu

Four genera and 10 species were collected from Jadu in this investigation. Noticeably, genus *Penicillium* harbored the highest number of species (5) and total count of colony (2180 out of 5480, 39.8%). The genus represented by *P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum*, *P. citrinum* and *P. puberulum* with the most common was *P. aurantiogriseum*. *Aspergillus* (1120 CFUs, 20.4% of total count) was reported in two species *A. aureolatus* (20.4%) and *A. deflectus* (14.6%). In addition, *Fusarium* was represented by two species (*F. oxysporum* and *F. solani*), it was constituting high number of colonies (36.6% of total fungal count in Jadu). The fourth genus, *Phialophora* was isolated as *P. richardisae* (3.3% of total CFUs).

4.1.4. Fungi isolated from Yafran

Nine species related to seven genera were reported here. Only genus *Penicillium* reported in 3 species (*P. aurantiogriseum*, *P. chrysogenum* and *P. expansum*) with 12.1% of total fungal colonies. The other remaining genera were represented by only one species. Regarding to the number of colonies forming units, *Mucor hiemalis* showed the highest (30.5%) followed by *Fusarium solani* (25.9%). The other species ranged from 2.8 to 14.2% of total count of fungi isolated from Yafran sample.

4.1.5. Fungi isolated from Rujban

Six genera presented by 11 species were recovered, regarding the number of species, genus *Penicillium* was the first. It was reported in 4 species with *P. crustosum*is the most common (140 colony out of 400 *Penicillium* colonies). It is worth mentioning that, genus*Fusarium* (*F.oxysporum* and *F. solani*) was recovered in high CFUs (1960, 45% of total count). *Aspergillus* was the same in the number of species (*A. aureolatus* and *A. niger*), both species harbored 13.8% of total count. The other remaining genera (3) were presented in one species each (*Geotrichum candidum*, *Mucor hiemalis* and *Trichoderma harzianum*) and CFUs ranging from 5.5 to 15.6% of the fungal count percentage.

4.1.6. Fungi isolated from Al-Asabaa

Four genera and 8 species were encountered in this sample. The most common genus was *Fusarium*, it was showed the highest count 40.7% of total fungal count (700 colonies out of 1720) and represented by 2 species (*F. oxysporum* and *F. solani*). *Aspergillus* follows *Fusarium* in number of colonies (represented by 2 species), it was encountered 29.1% of the total count. As shown in table (1), *Penicillium* was the most diverse genus, it was represented by 3 species. It was harbored 15.1% of total fungal count isolated from Al-Asabaa**.**

4.1.7. Fungi isolated from Gharyan

Five genera including 11 species were totally collected from this sample. The most diverse genus was *Aspergillus*, recovered in 6 species, giving rise 55.8% of the total fungi. *A. fumigatus* the most common species, it was presented in 16.7% of *Aspergillus* count. *Penicillium* was reported in two species, *P. aurantiogriseum* and *P. griseofulvum*, giving rise 7.5% of the total fungi. The remaining species; *Fusarium solani*, *Mucor hiemalis* and *Myrothecium verrucaria* were emerged in 20.83, 8.34 and 7.5% of the total fungi.

Table 1. CFUs (Colony forming units) and CFUs% of fungi recovered from different samples of olive oil processing wastes (Fatura).

Fungal species	Zintan		Rayaina		Jadu		Yafran		Rujban		Al-Asabaa		Gharyan		Gross total	
	CFU	%	CFU	%	CFU	%	CFU	%	CFU	%	CFU	%	CFU	%	CFU	%CFU
***Alternaria* spp.**	**320**	**5.61**													**320**	**1.1**
A. alstroemeriae	200	3.51													200	0.7
A. angustiovoidea	120	2.1													120	0.4
***Aspergillus* spp.**	**1500**	**26.3**	**2020**	**47.42**	**1120**	**20.4**	**440**	**7.8**	**600**	**13.8**	**500**	**29.1**	**1340**	**55.83**	**7520**	**25.44**
A. aureolatus	400	7.0	600	14.1	320	5.8	440	7.8	200	4.6			240	10	2200	7.4
A. caespitosus											200	11.2	220	9.2	420	1.4
A. deflectus			120	2.8	800	14.6									920	3.1
A. fumigatus			800	18.8									400	16.7	1200	4.0
A. niger	580	10.2							400	9.2			140	5.83	1120	3.8
A. silvaticus													280	11.6	280	0.94
A. terreus											300	17.4			300	1.0
A. ustus	520	9.1	500	11.7									60	2.5	1080	3.65
***Fusarium* spp.**	**2020**	**35.44**	**380**	**8.91**	**2000**	**36.5**	**1460**	**25.9**	**1960**	**44.9**	**700**	**40.7**	**500**	**20.83**	**9020**	**30.5**
F. oxysporum					120	2.2			160	3.6	260	15.1			540	1.8
F. solani	2020	35.4	380	8.91	1880	34.3	1460	25.9	1800	41.3	440	25.6	500	20.83	8480	28.7
Geotrichum candidum									**240**	**5.5**					**240**	**0.8**
Humicola fusco-atra							**800**	**14.2**							**800**	**2.7**
Microdochium nivale	**120**	**2.11**													**120**	**0.4**
***Mucor* spp.**	**880**	**15.44**	**520**	**12.21**			**1720**	**30.5**	**680**	**15.6**			**380**	**15.84**	**4180**	**14.14**
M. hiemalis	880	15.44	40	0.94			1720	30.5	680	15.6			200	8.34	3520	11.9
M. racemosus			360	8.45											360	1.2
Mucor sp.			120	2.8											120	0.4
Myrothecium verrucaria													**180**	**7.5**	**180**	**0.6**
***Penicillium* spp.**	**660**	**11.6**	**780**	**18.31**	**2180**	**39.8**	**680**	**12.1**	**400**	**9.2**	**260**	**15.1**	**180**	**7.5**	**5140**	**17.4**
P. aurantiogriseum			180	4.2	1000	18.2	280	4.9	100	2.3	120	7.0	60	2.5	1740	5.8
P. brevicompactum			160	3.75	120	2.2									280	0.94
P. chrysogenum			180	4.2	600	10.9	200	3.6			100	5.8			1080	3.65
P. citrinum	60	1.0	260	6.1	260	4.7			80	1.8	40	2.3			700	2.36
P. crustosum									140	3.2					140	0.47
P. expansum							200	3.6							200	0.67
P. griseofulvum									80	1.8			120	5	200	0.67
P. puberulum					200	3.6									200	0.67
P. roqueforti	600	10.5													600	2.0
Phialophora richardisae	**200**	**3.5**			**180**	**3.3**	**160**	**2.8**							**540**	**1.82**
Stachybotrys chartarum			**560**	**13.15**											**560**	**1.9**
Trichoderma harzianum							**380**	**6.7**	**480**	**11.0**	**260**	**15.1**			**1120**	**3.8**
Total	**5700**	**100**	**4260**	**100**	**5480**	**100**	**5640**	**100**	**4360**	**100**	**1720**	**100**	**2400**	**100**	**29560**	**100**
No. of genera	**7**		**5**		**4**		**7**		**6**		**4**		**5**		**12**	
No. of species	**11**		**13**		**10**		**9**		**11**		**8**		**11**		**31**	

The isolated fungal species are summarized below (Figures 4-34).

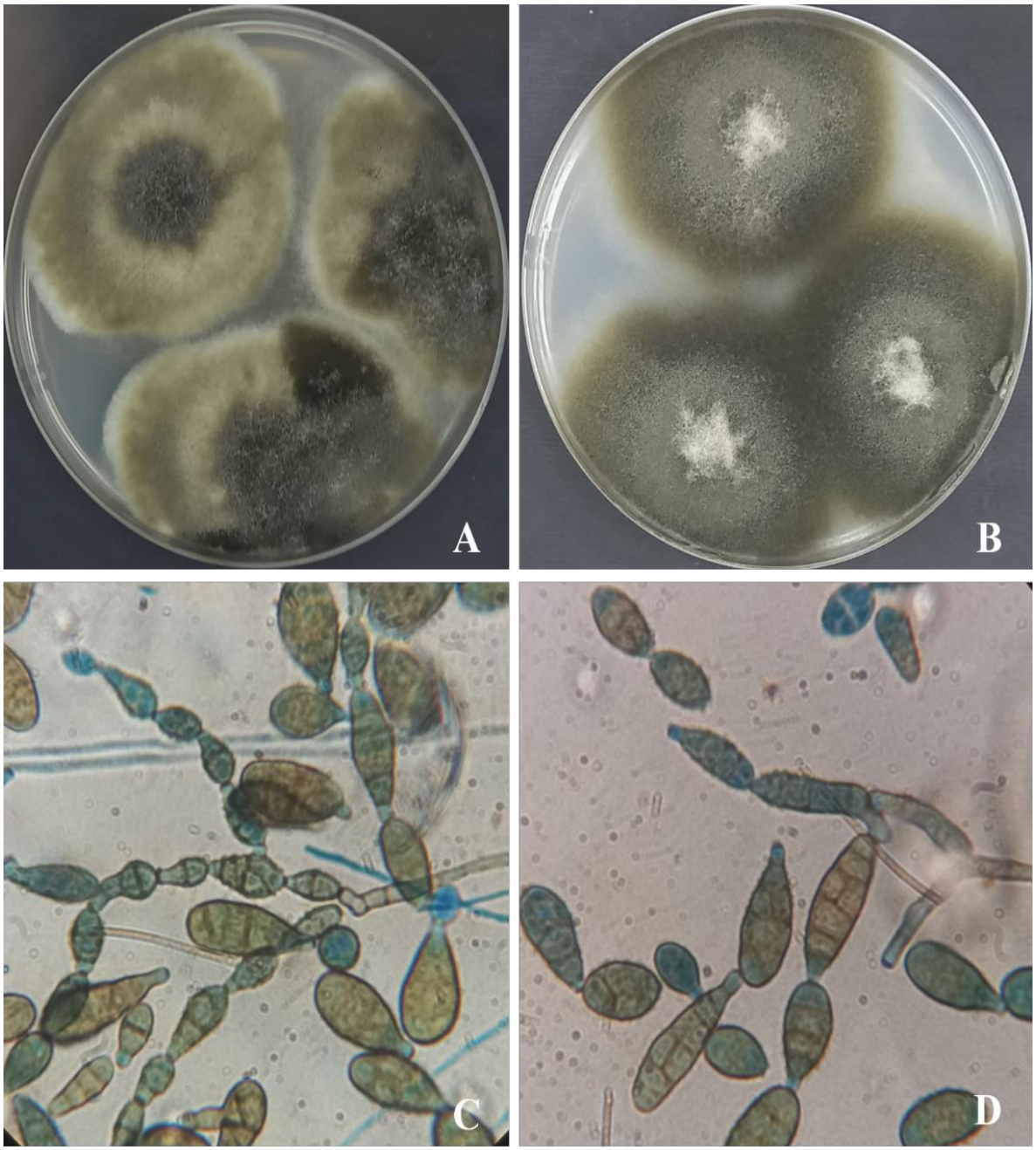

Figure 4. ***Alternaria alstroemeriae*AUMC 16060:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, chains of conidia.

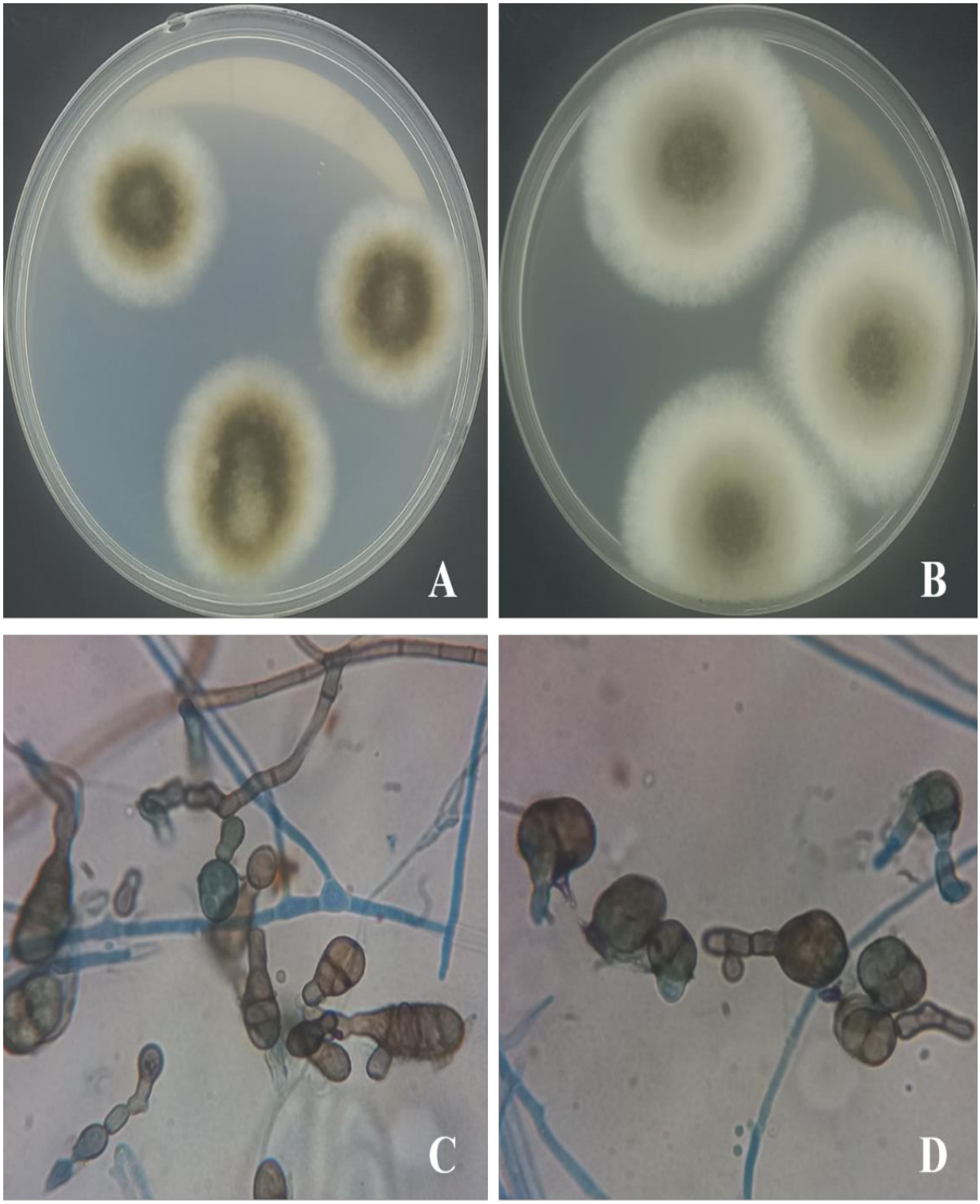

Figure 5. ***Alternaria angustiovoidea*** **AUMC 16062:**A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, chains of conidia.

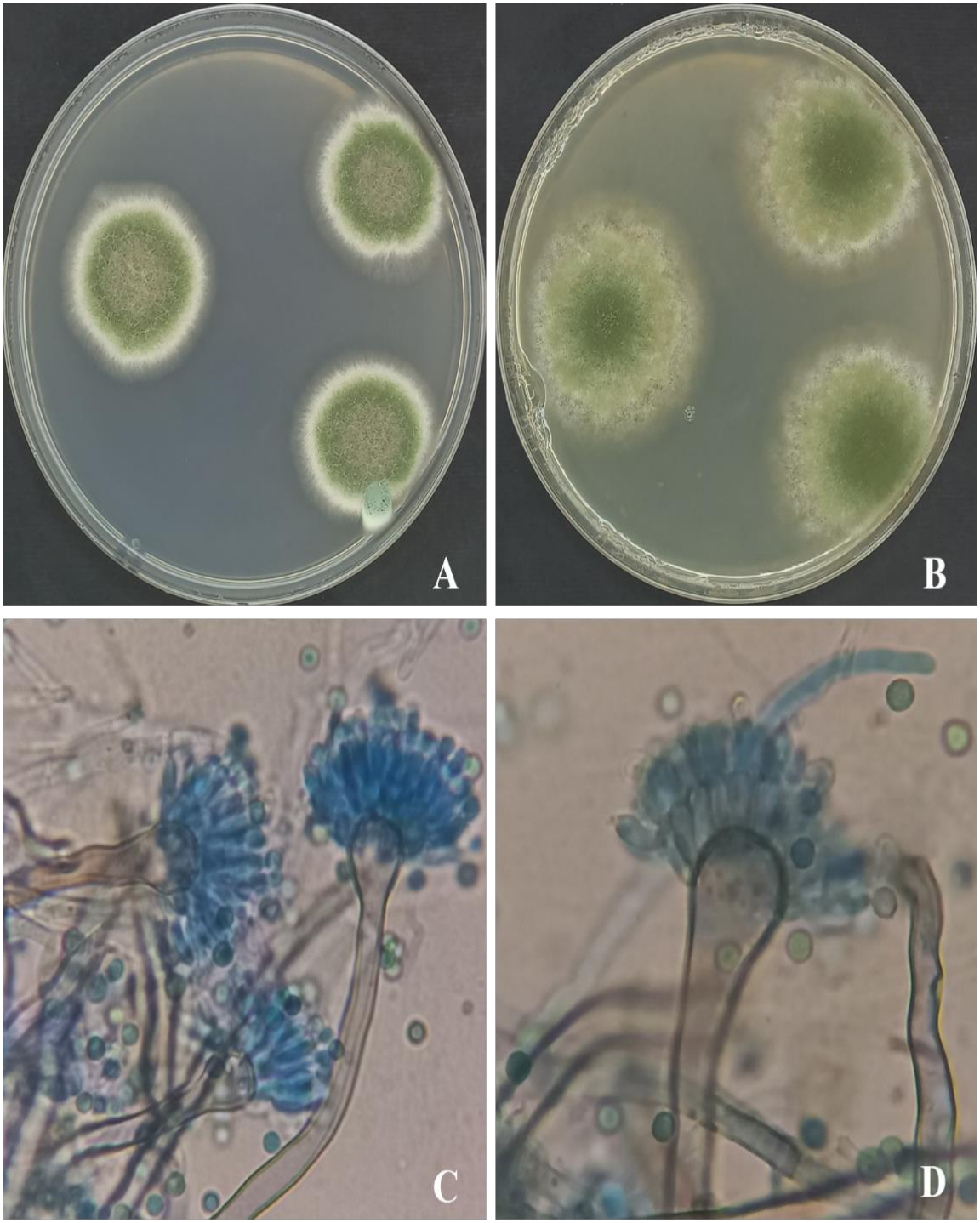

Figure 6. ***Aspergillus aureolatus*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and biseriate conidial heads.

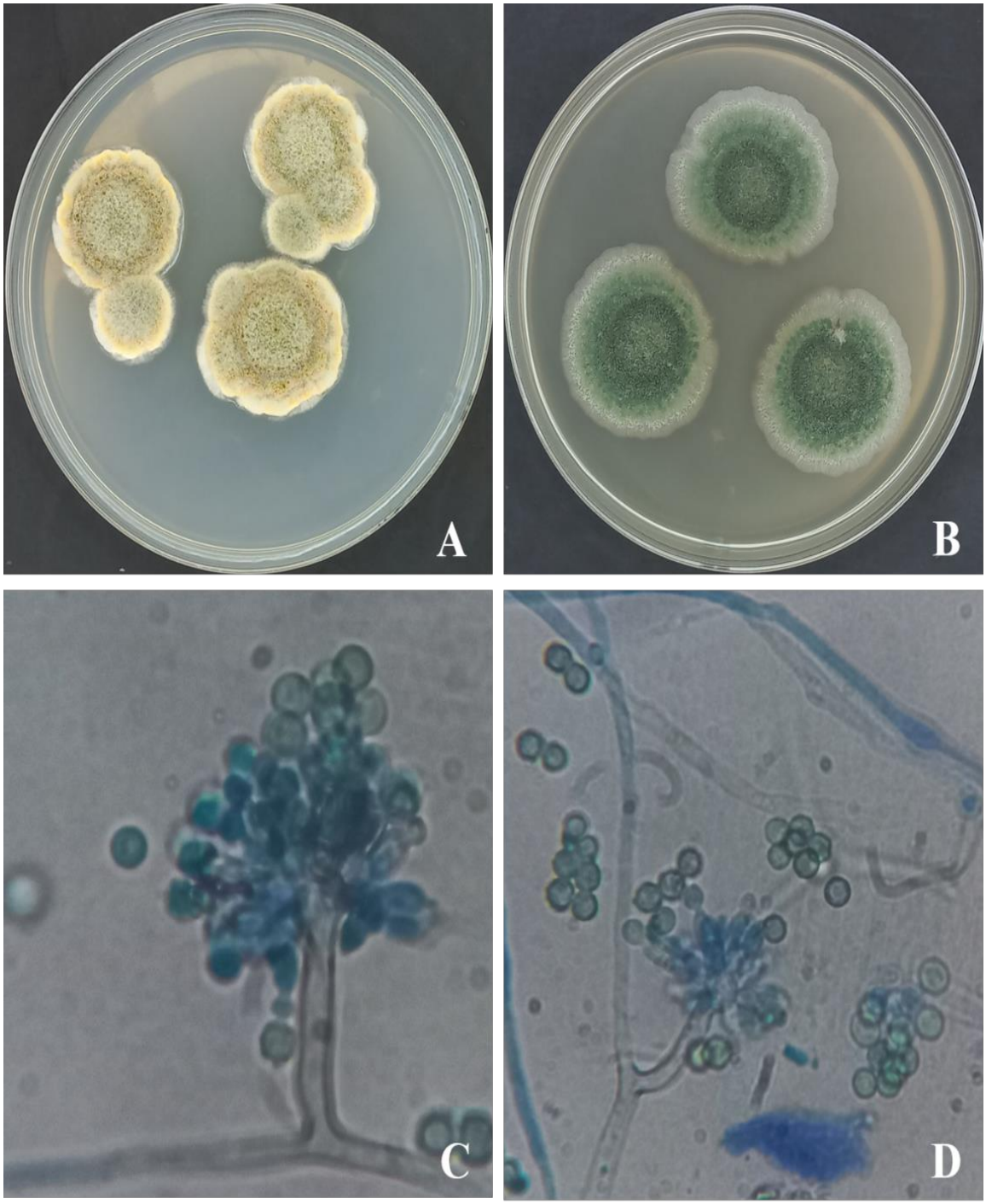

Figure 7. ***Aspergillus caespitosus*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and biseriate conidial heads.

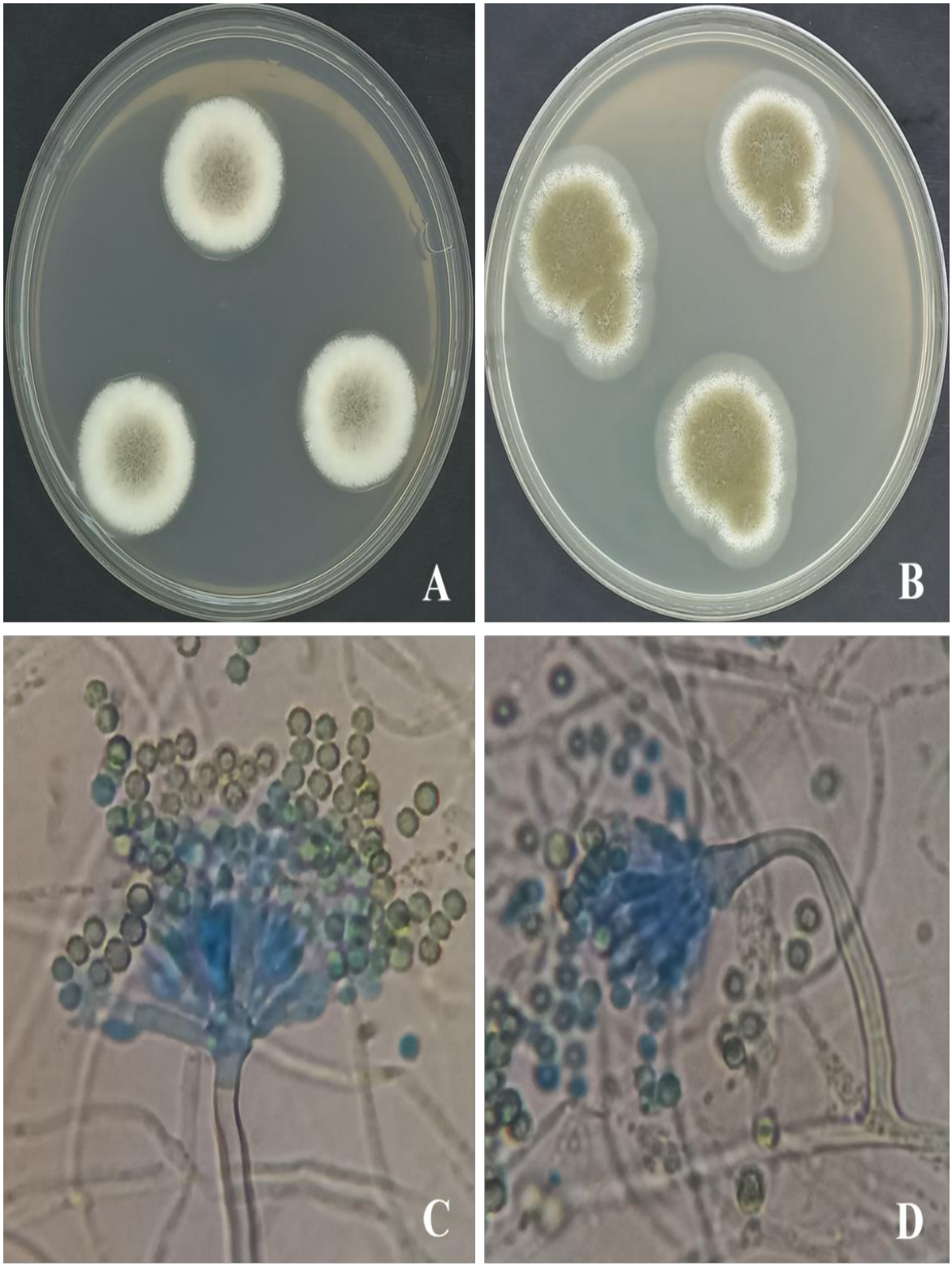

Figure 8. ***Aspergillus deflectus*:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and biseriate conidial heads with rough-walled conidia.

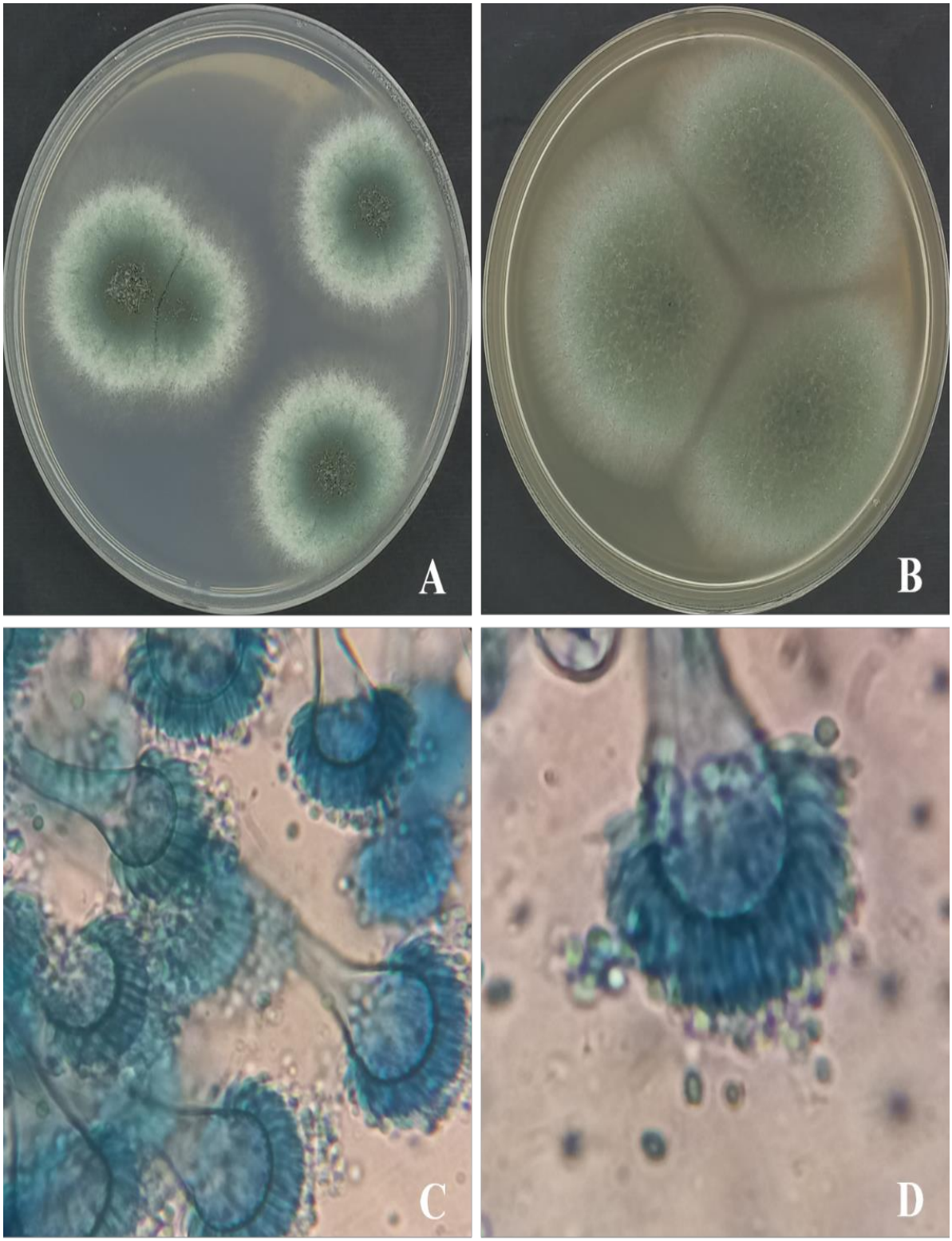

Figure 9. ***Aspergillus fumigatus*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and uniseriate, columnar conidial heads and elliptical conidia.

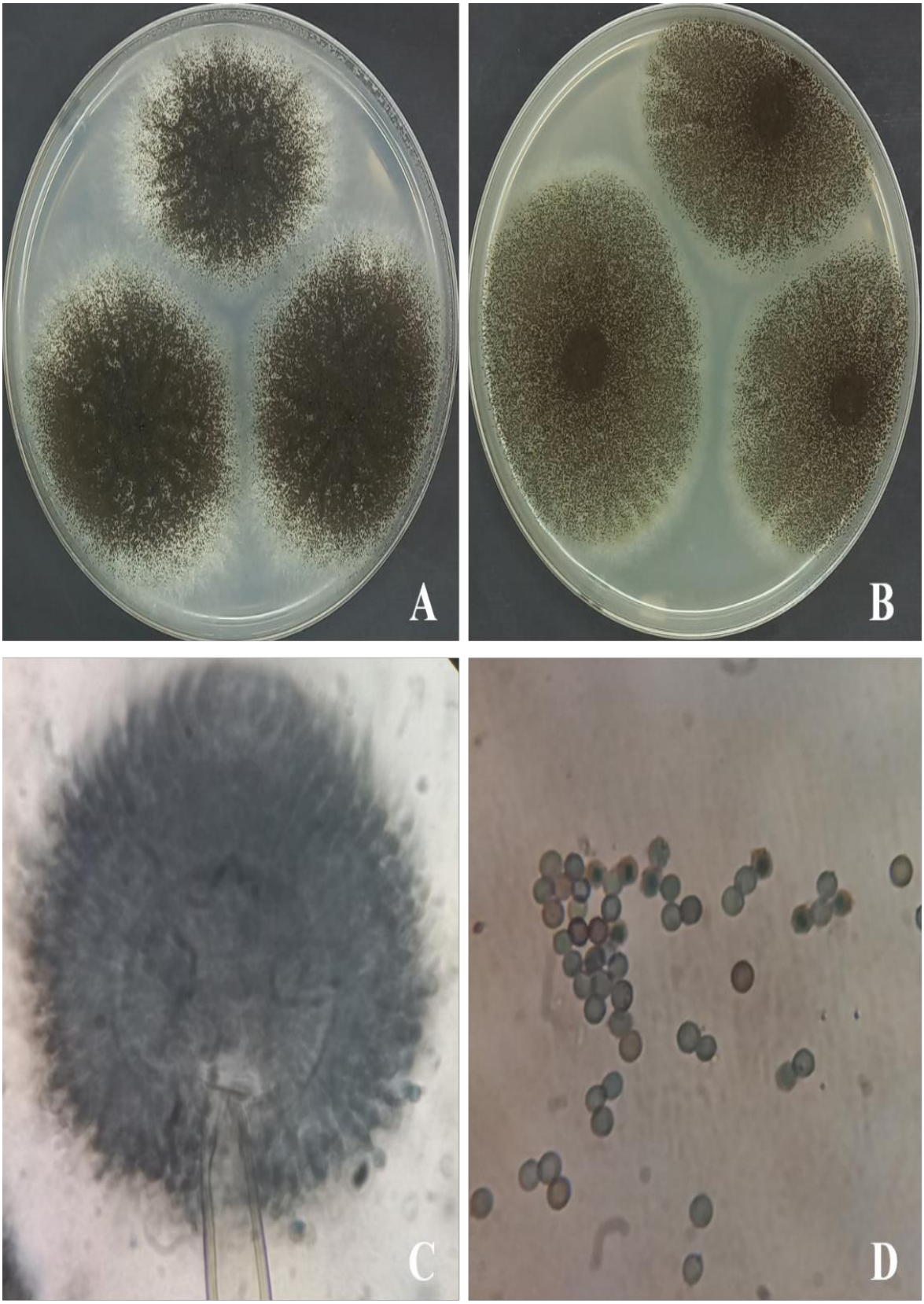

Figure10. ***Aspergillus niger*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C, Conidiophores and biseriate conidial heads. D, conidia.

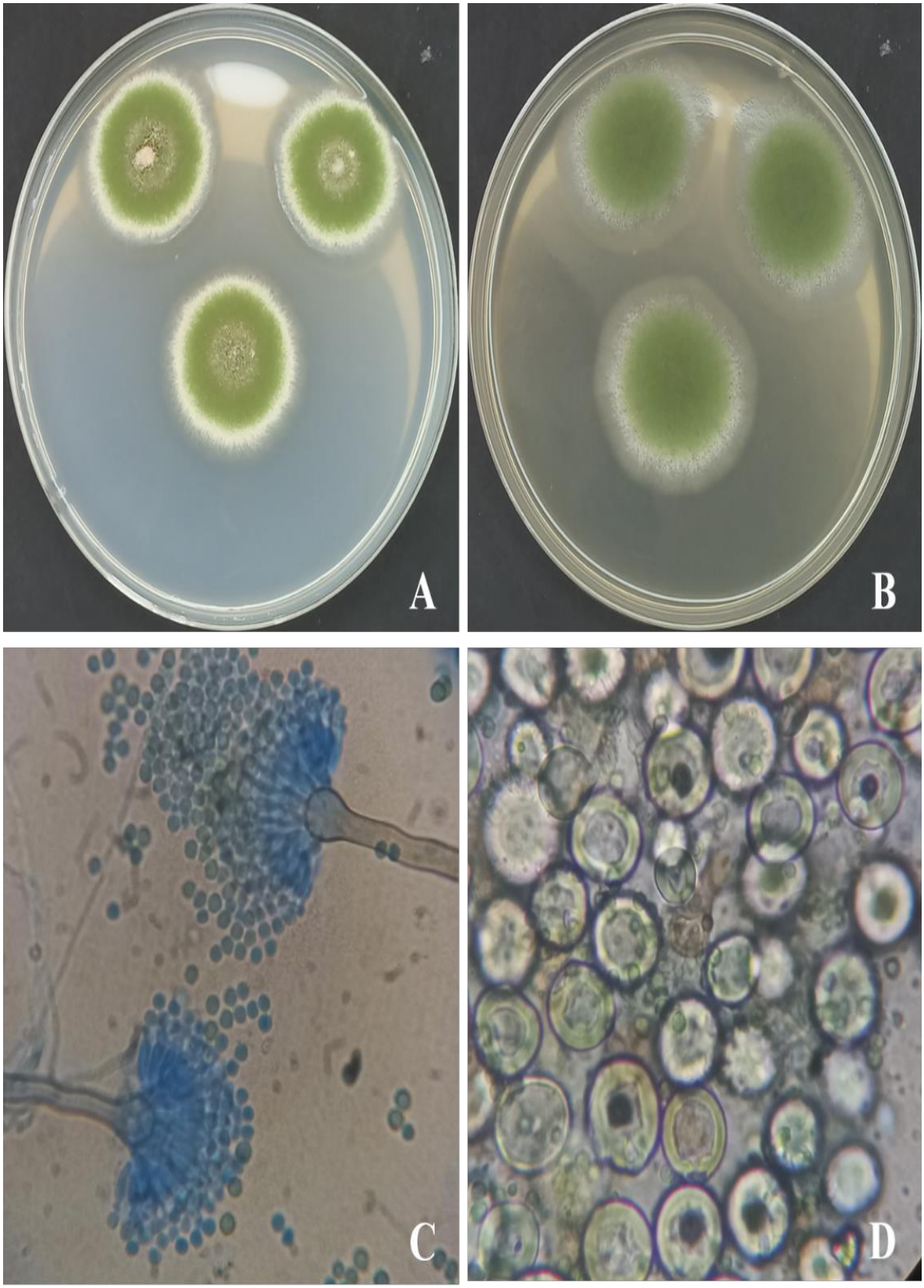

Figure11.*Aspergillus silvaticus*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C, Conidiophores with biseriate conidial heads. D, Hulle cells.

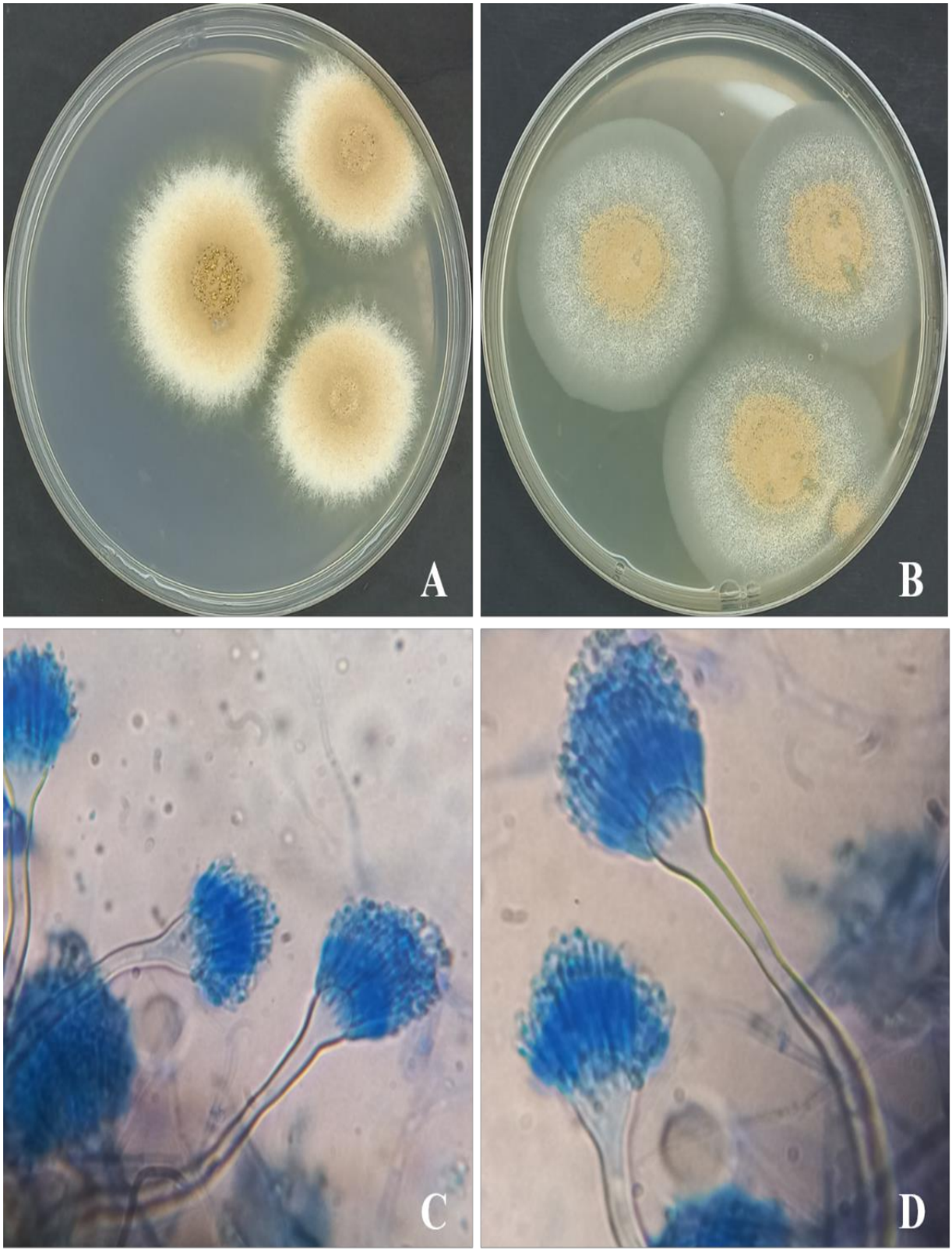

Figure12. ***Aspergillus terreus*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and biseriate, columnar conidial heads.

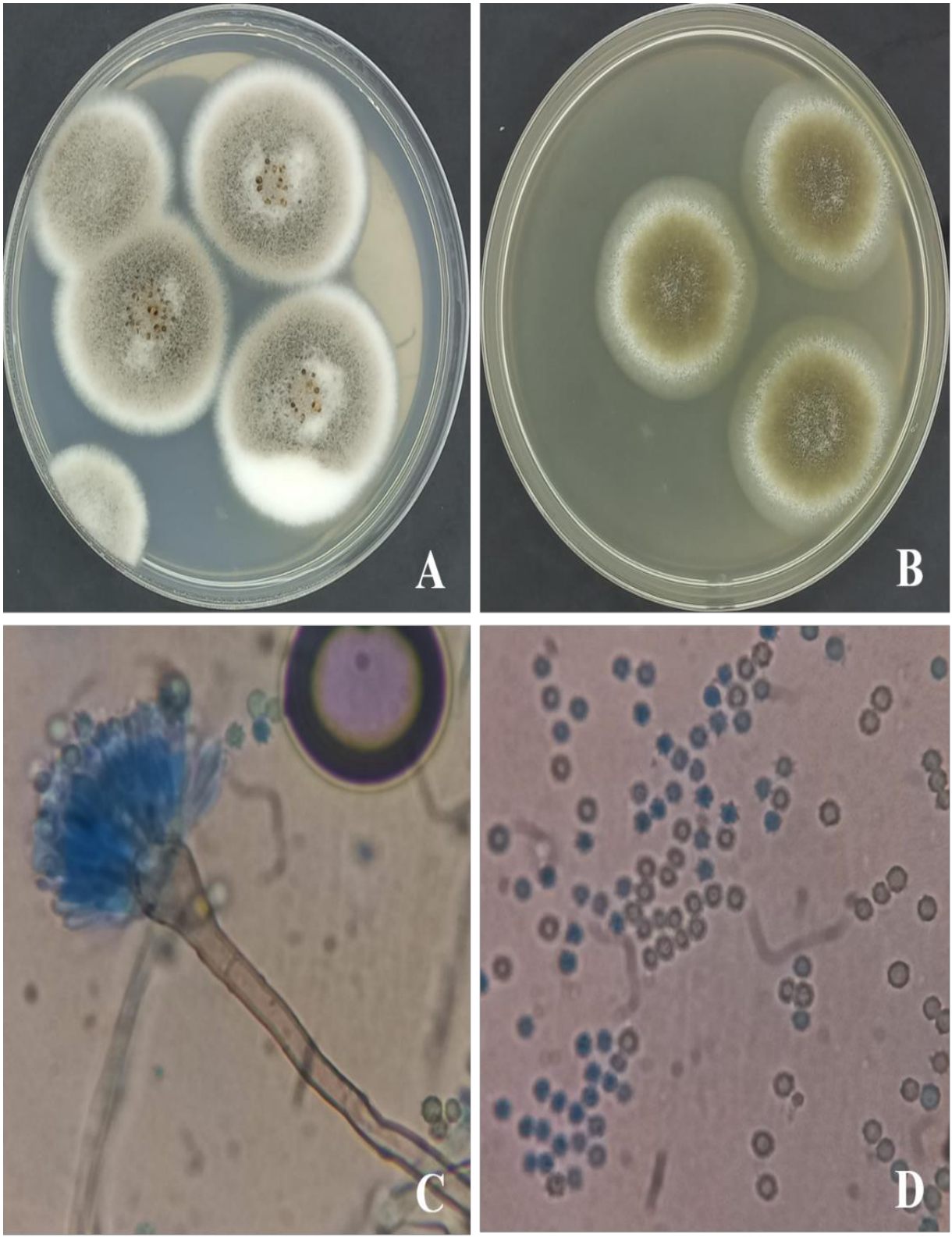

Figure13. ***Aspergillus ustus*****:** A-B, Seven-day-old colonies on .Cz and MEA at 25°C. C, Conidiophores and biseriate, columnar conidial heads. D, Conidia.

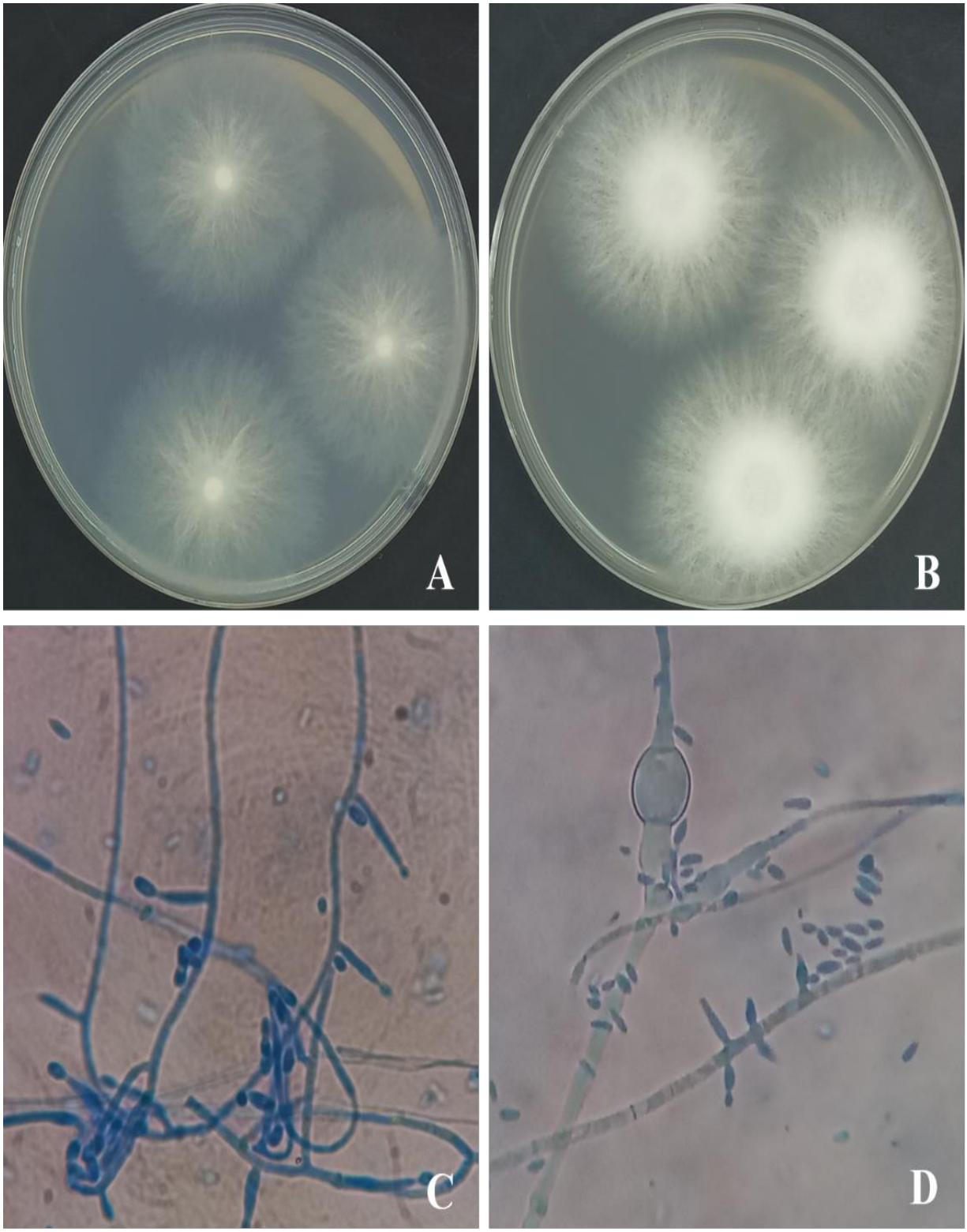

Figure14. ***Fusarium oxysporum*****:** A-B, Seven-day-old colonies on Cz and PDA at 25°C. C-D, Conidiophores and short phialides.

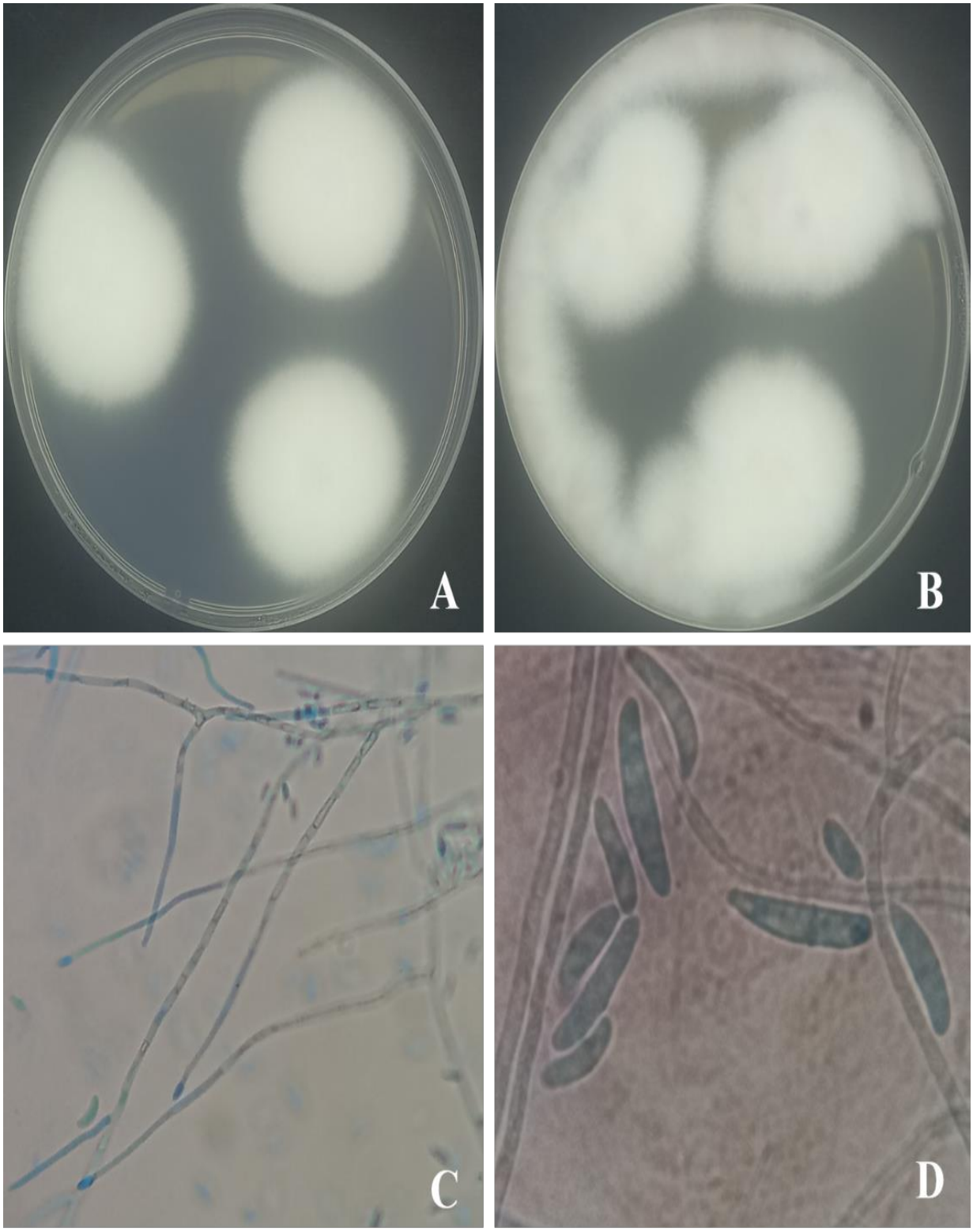

Figure15. *Fusarium solani*: A-B, Seven-day-old colonies on Cz and PDA at 25°C. C, Long phialides bearing microconidia. D, Falcate macroconidia.

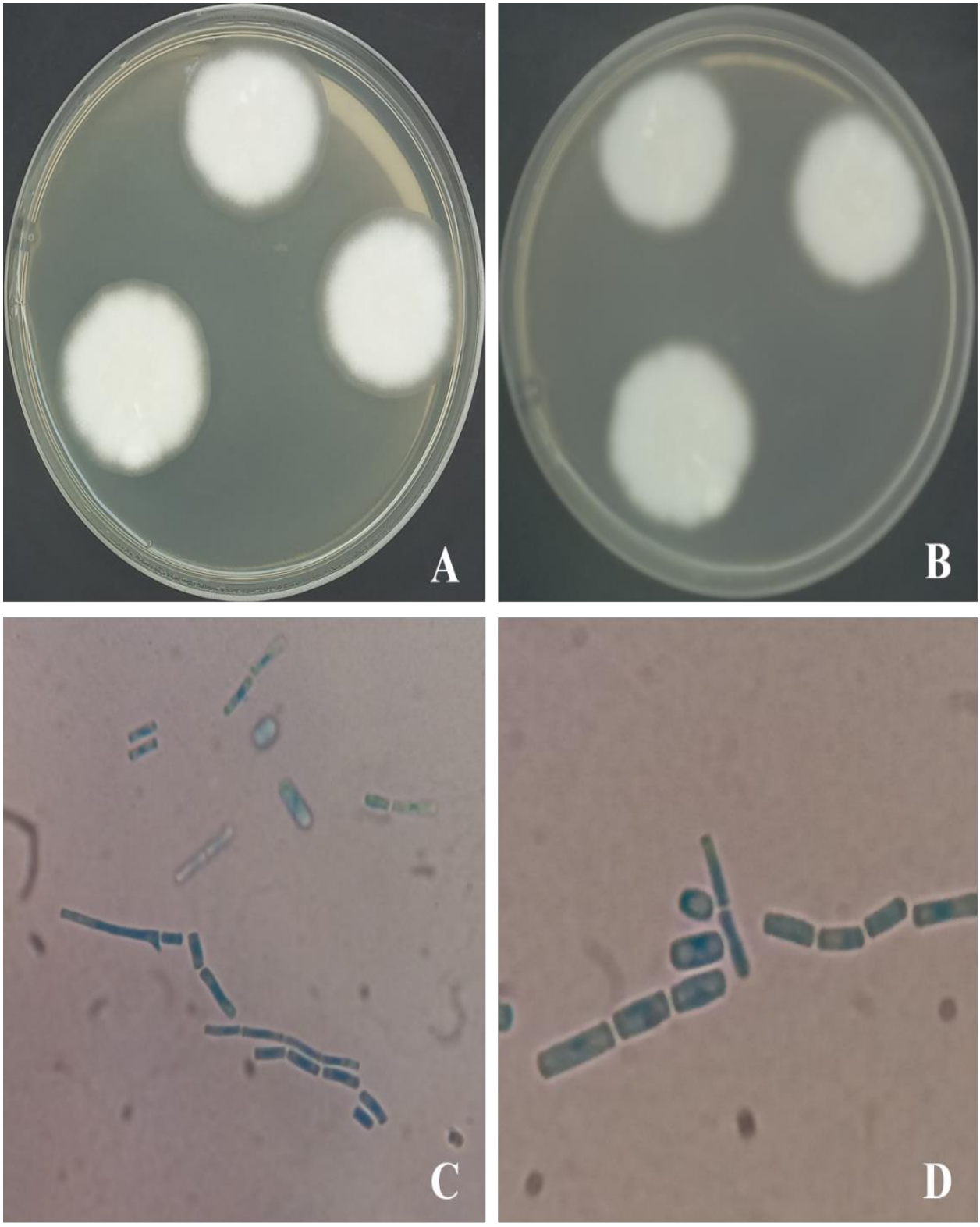

Figure16. *Geotrichum candidum*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Chains of arthroconidia.

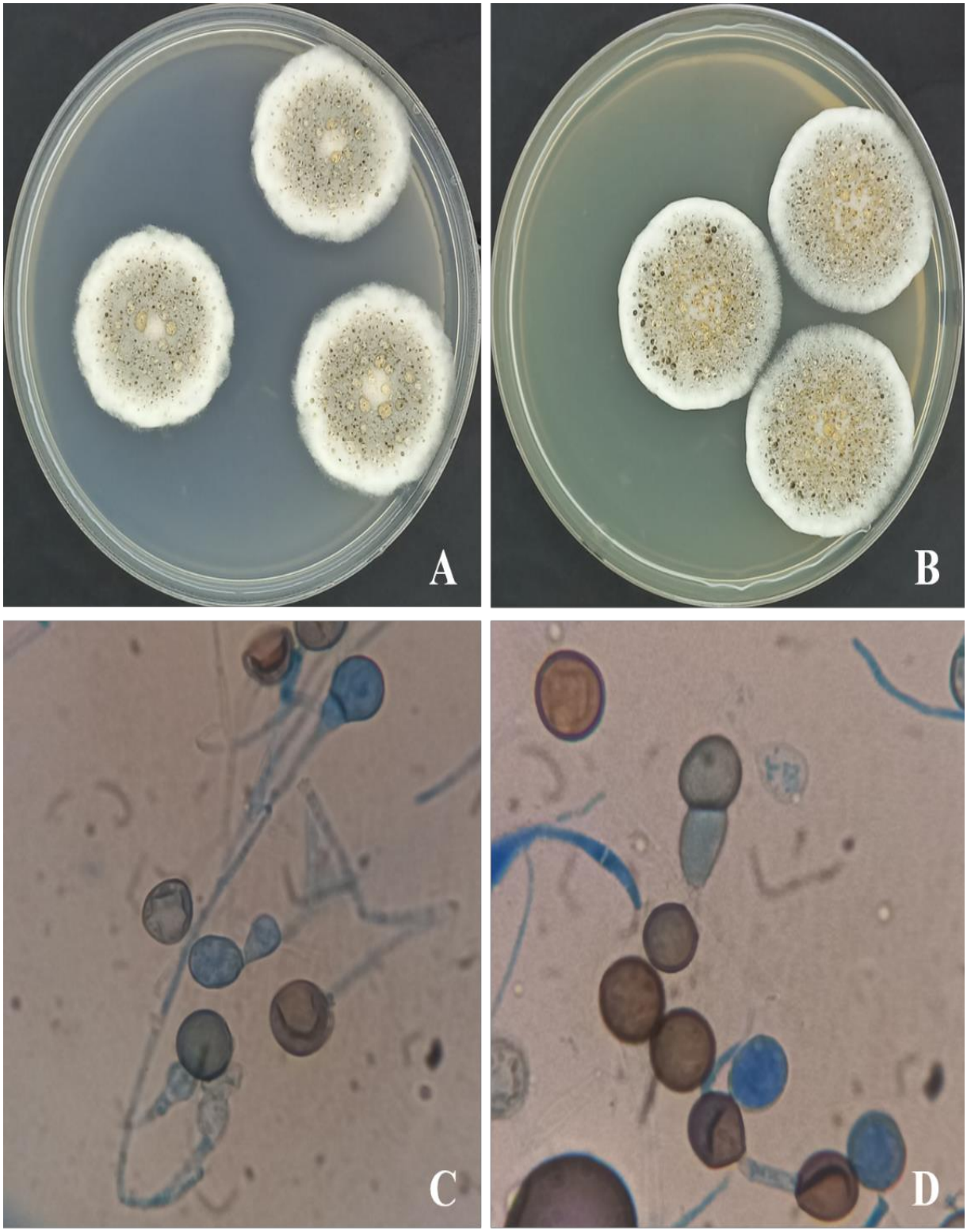

Figure17. *Humicola fusco-atra*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Phialides and conidia.

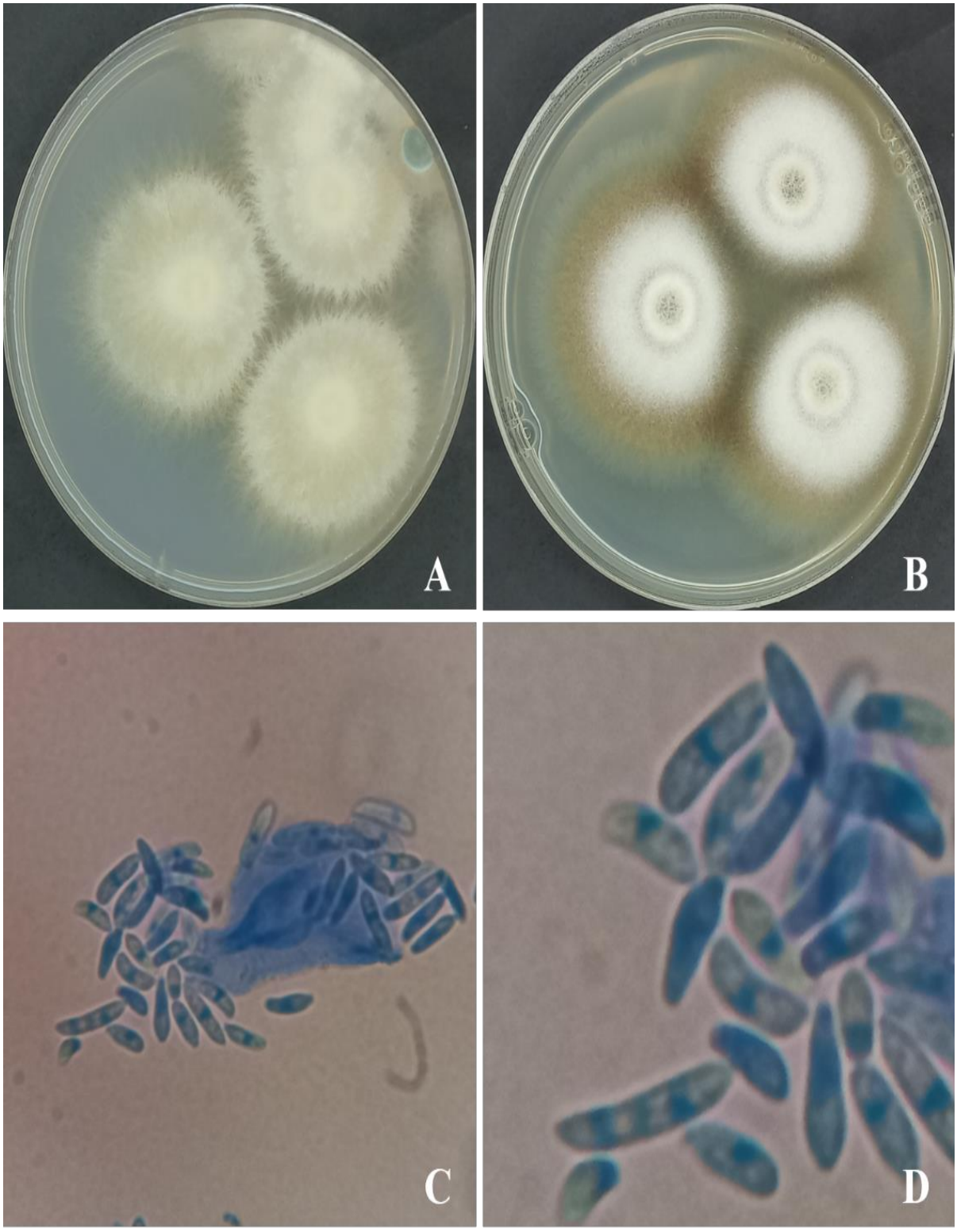

Figure18. ***Microdochium nivale*****:** A-B, Seven-day-old colonies on Cz and PDA at 25°C. C-D, Conidia.

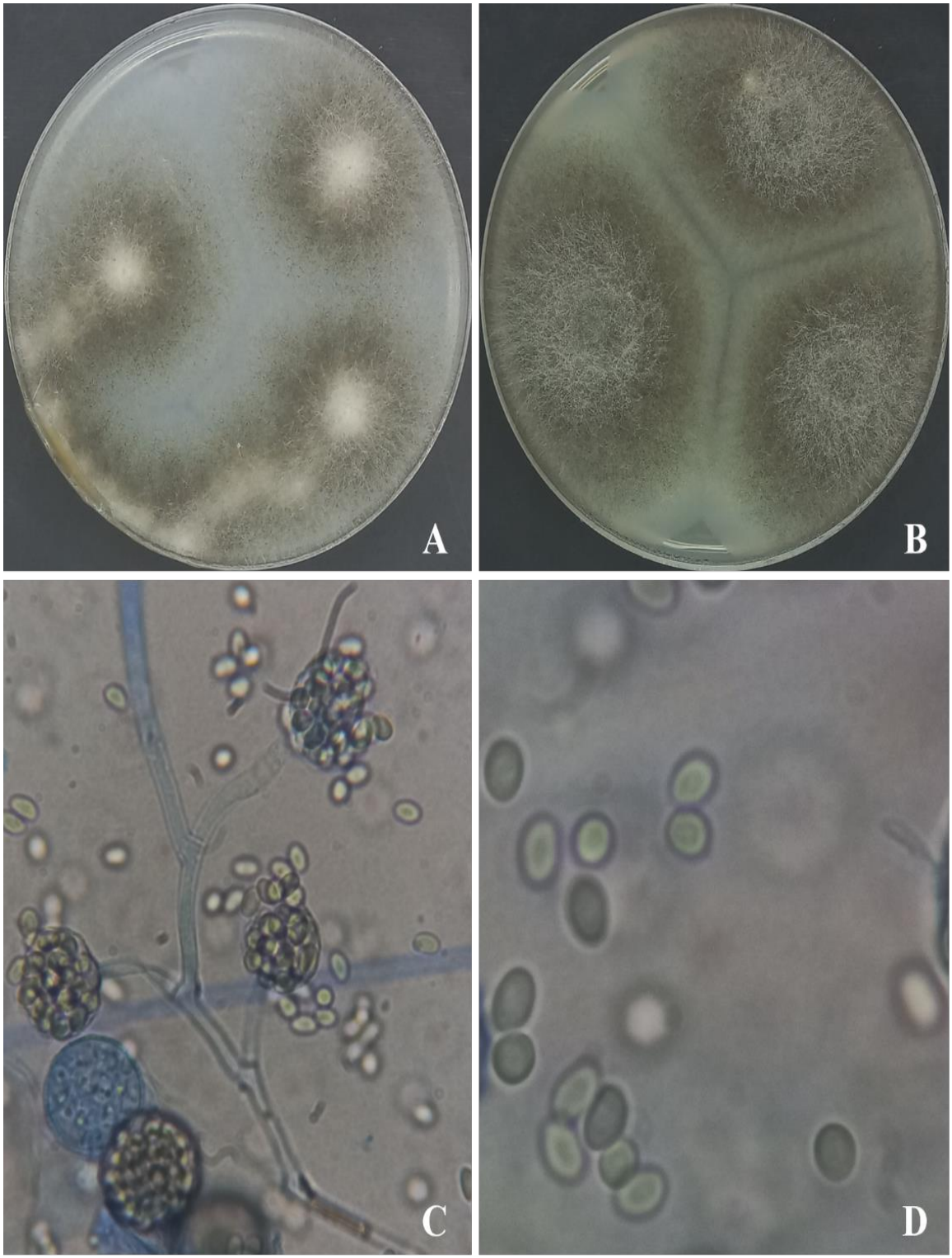

Figure19. ***Mucor hiemalis*****:** A-B, Seven-day-old colonies on Cz and PDA at 25°C. C, Sporangiophores. D, Sporangiospores.

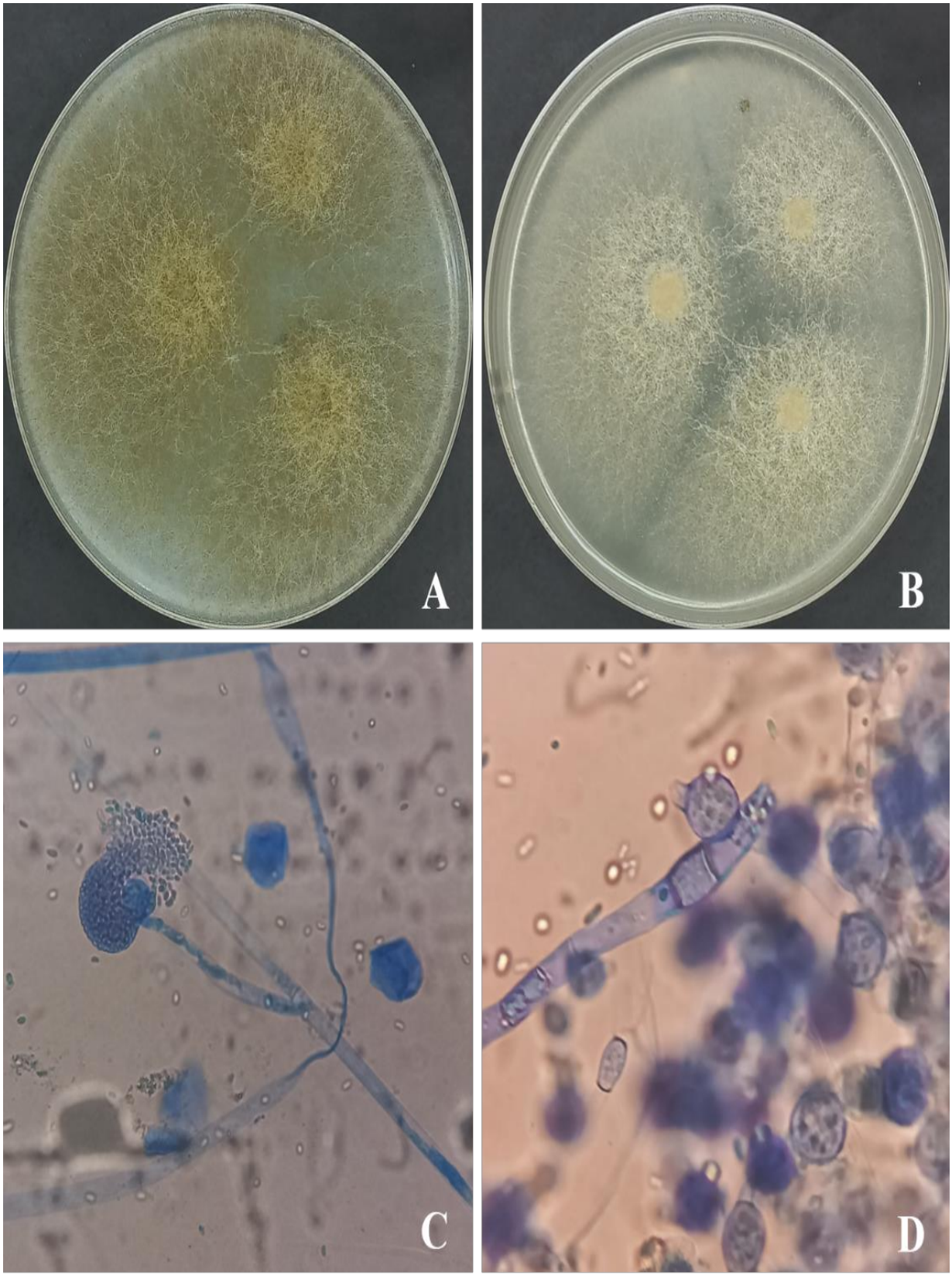

Figure 20. ***Mucor racemosus*****:** A-B, Seven-day-old colonies on Cz and PDA at 25°C. C, Sporangiophores. D, Sporangiospores.

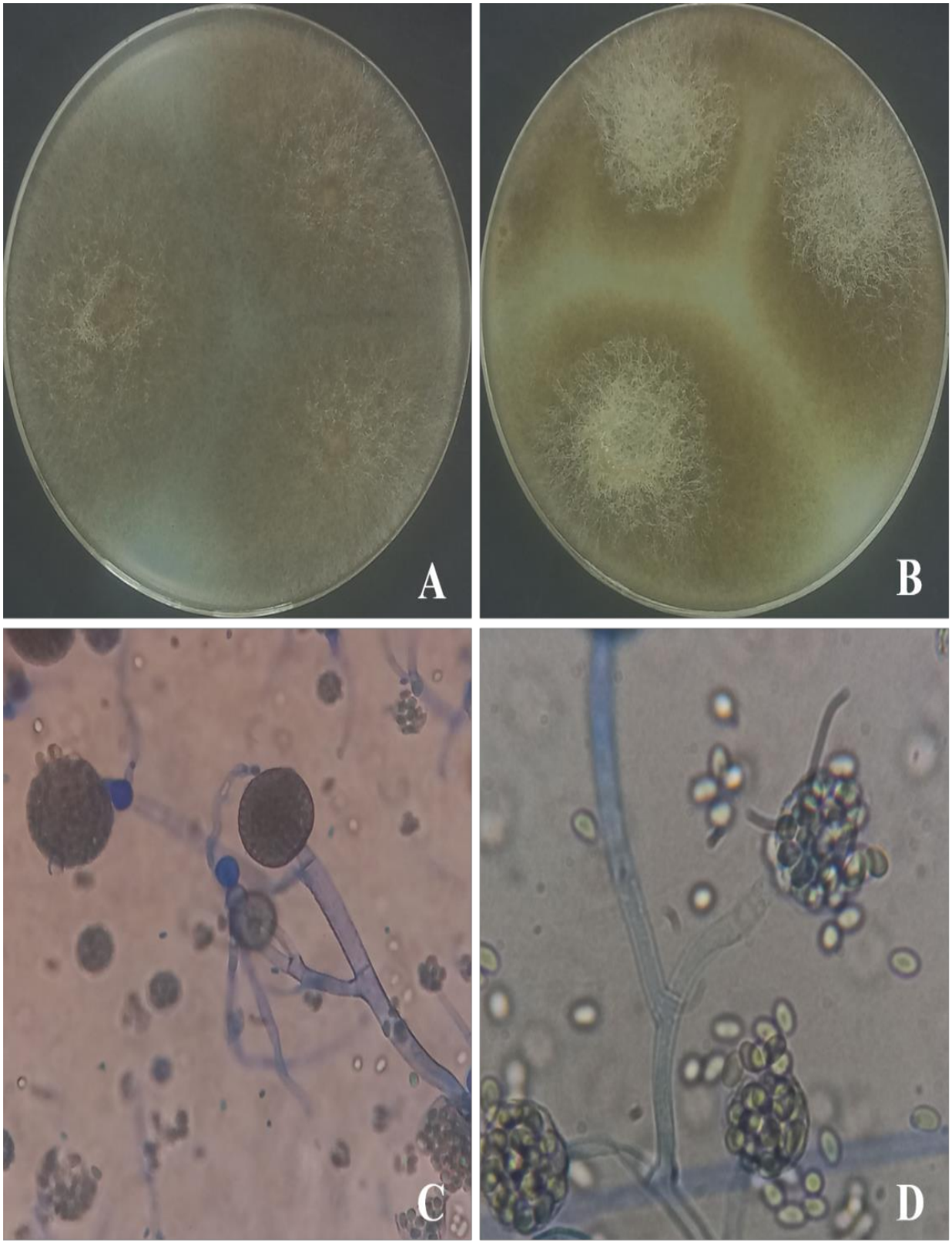

Figure 21. ***Mucor*** **sp.:** A-B, Seven-day-old colonies on Cz and PDA at 25°C. C, Sporangiophores. D, Sporangiospores.

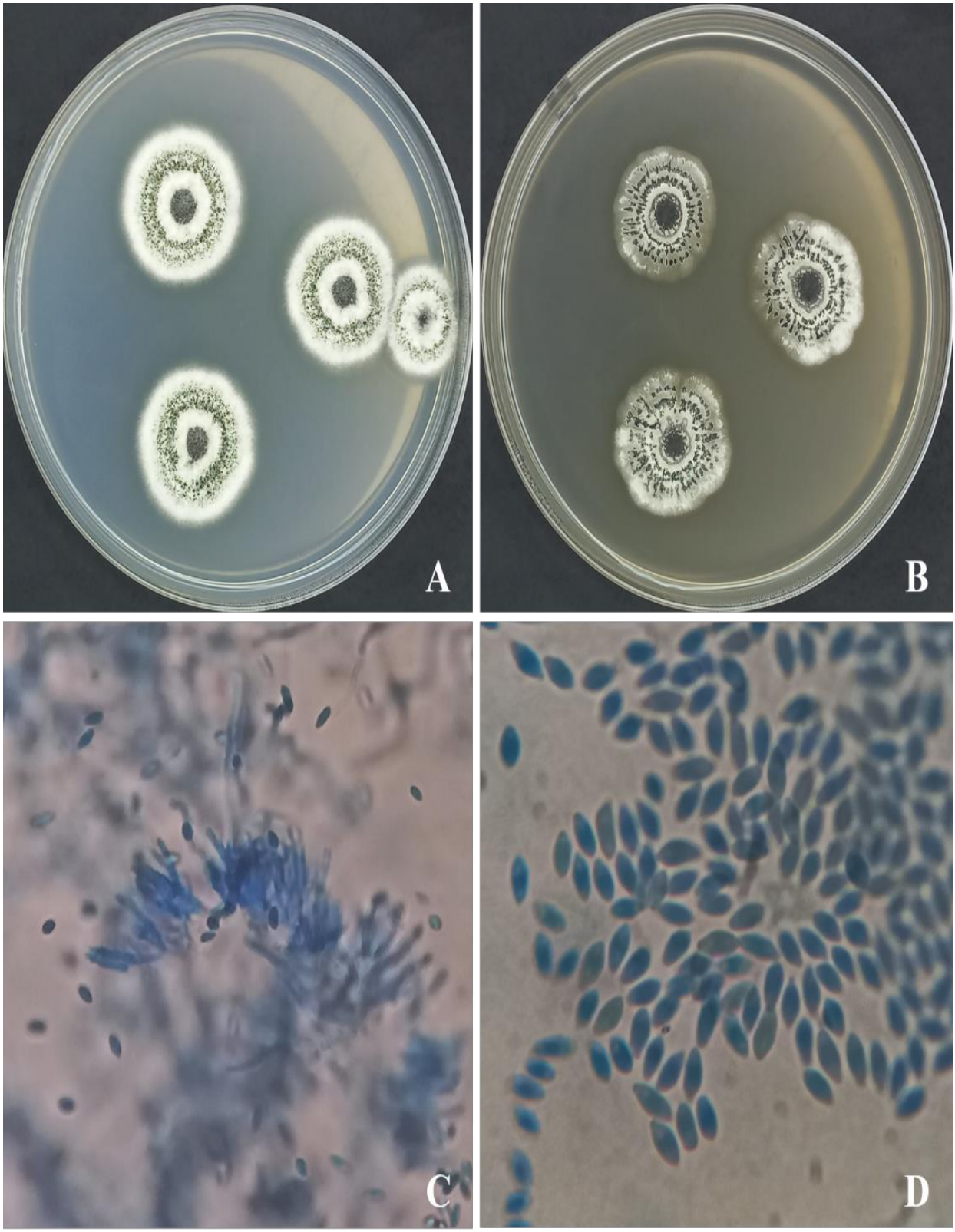

Figure22. ***Myrothecium verrucaria*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C, Sporodochium. D, Conidia.

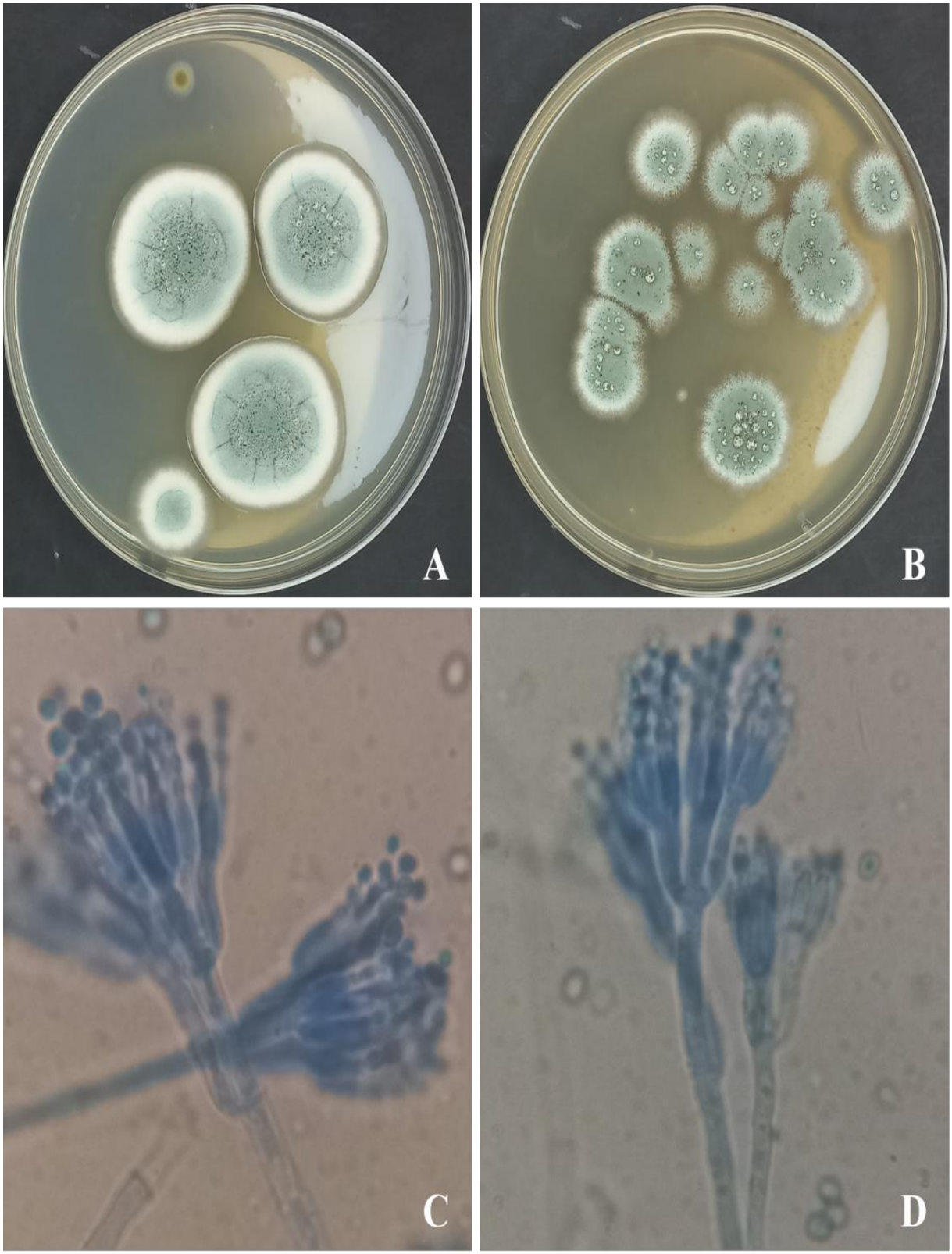

Figure23. ***Penicillium aurantiogriseum*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and terverticillate penicilli.

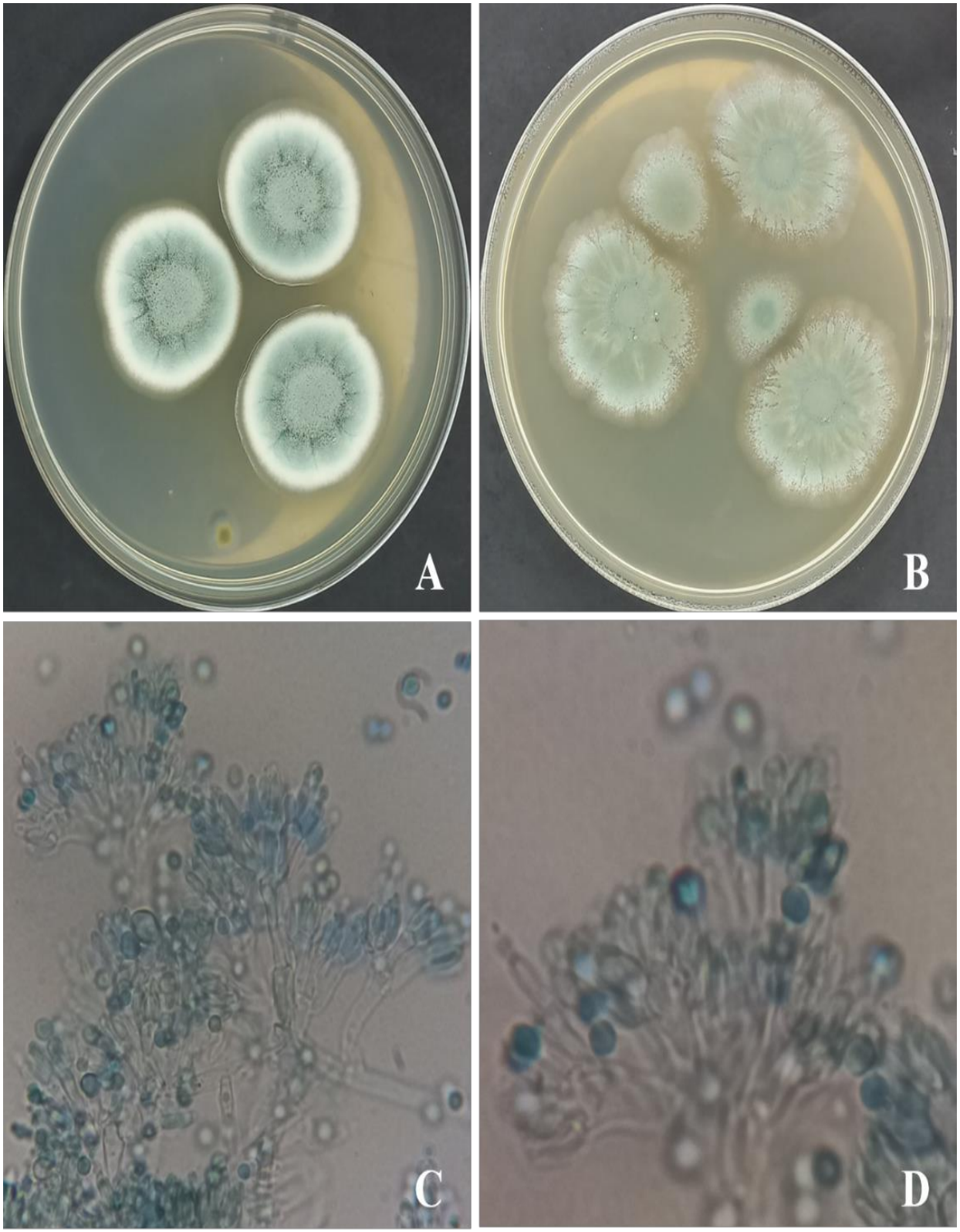

Figure24. ***Penicillium brevicompactum*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and short, compact phialides.

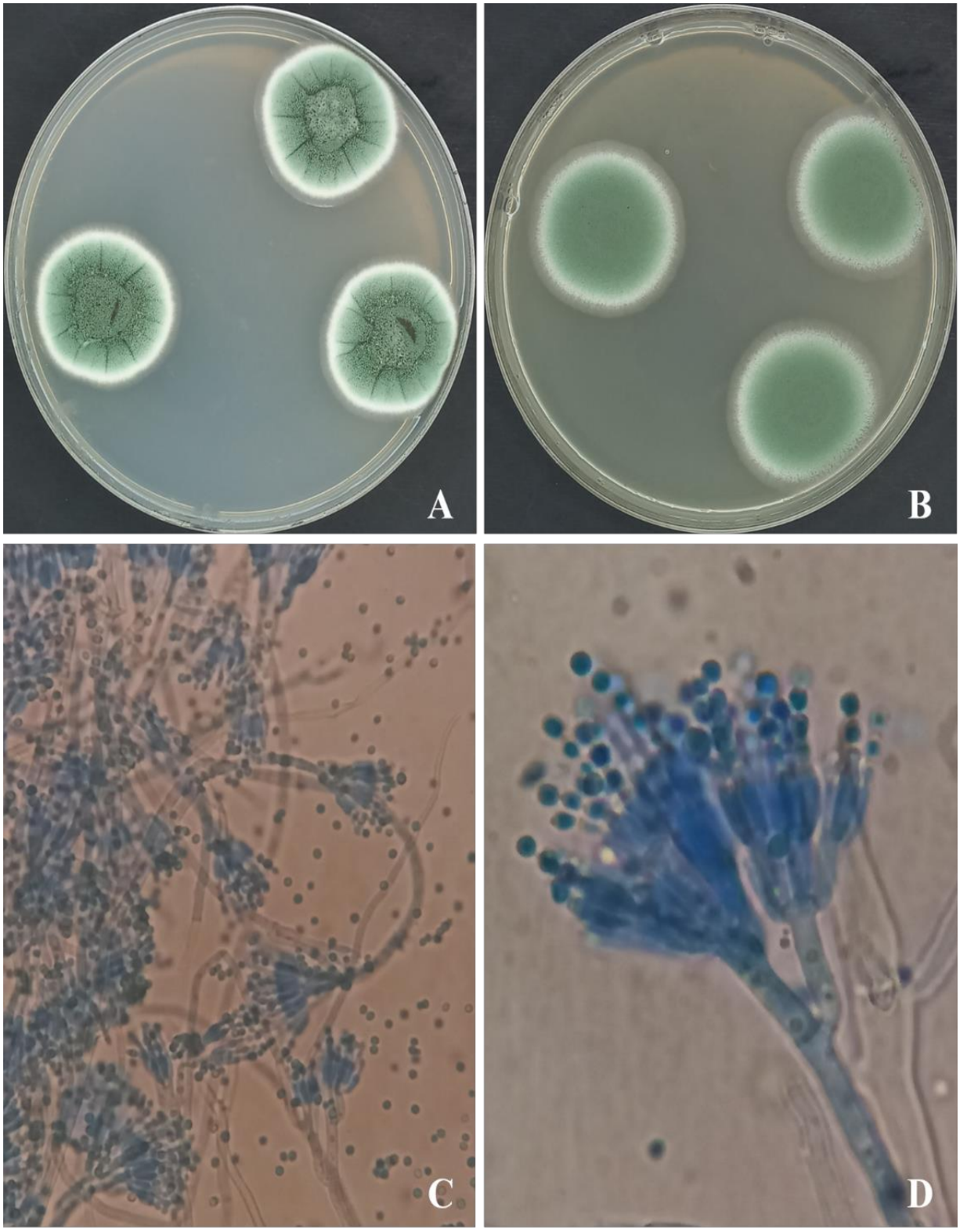

Figure25. ***Penicillium chrysogenum*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and terverticillate penicilli.

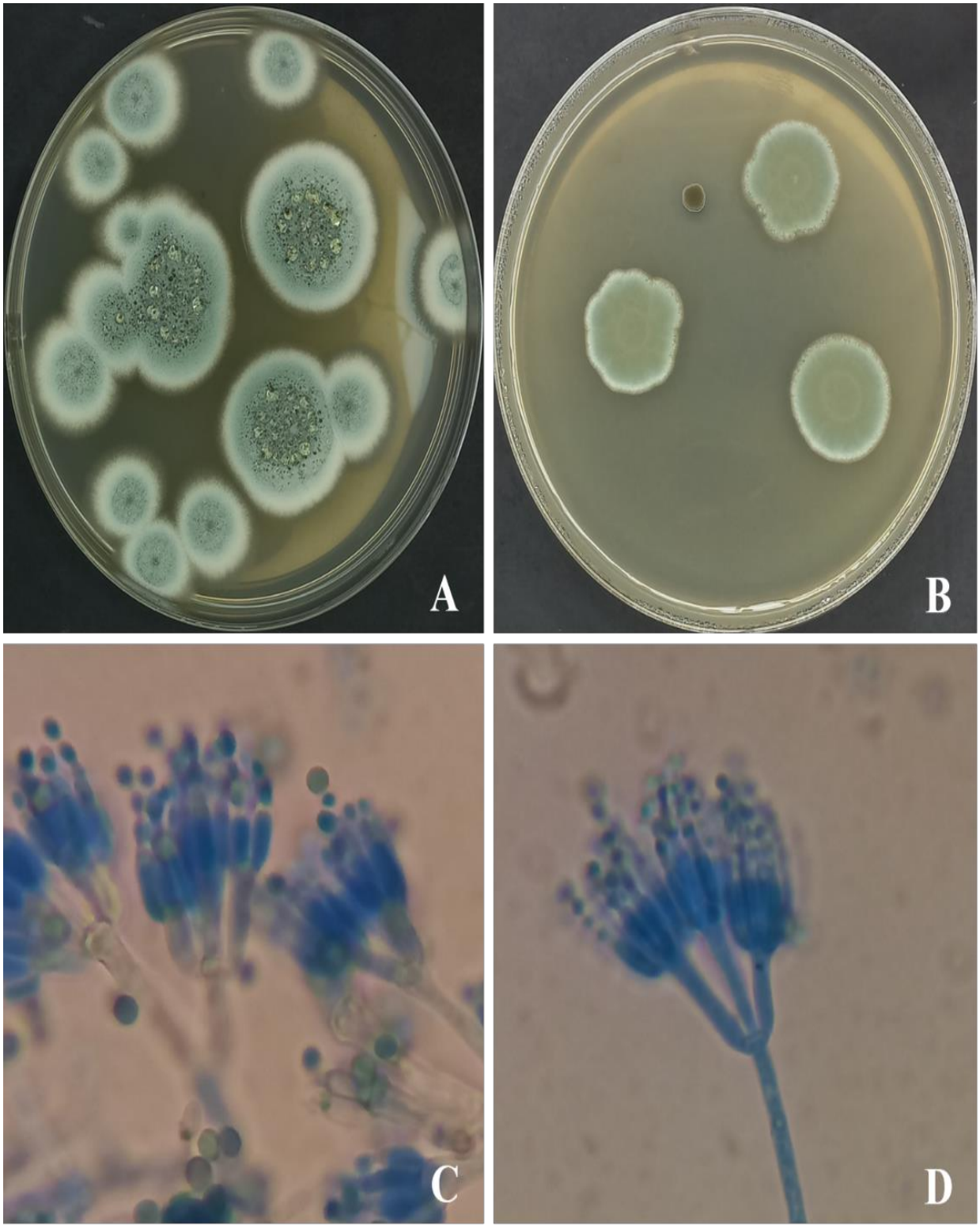

Figure26. *Penicillium citrinum*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and biverticillate penicilli.

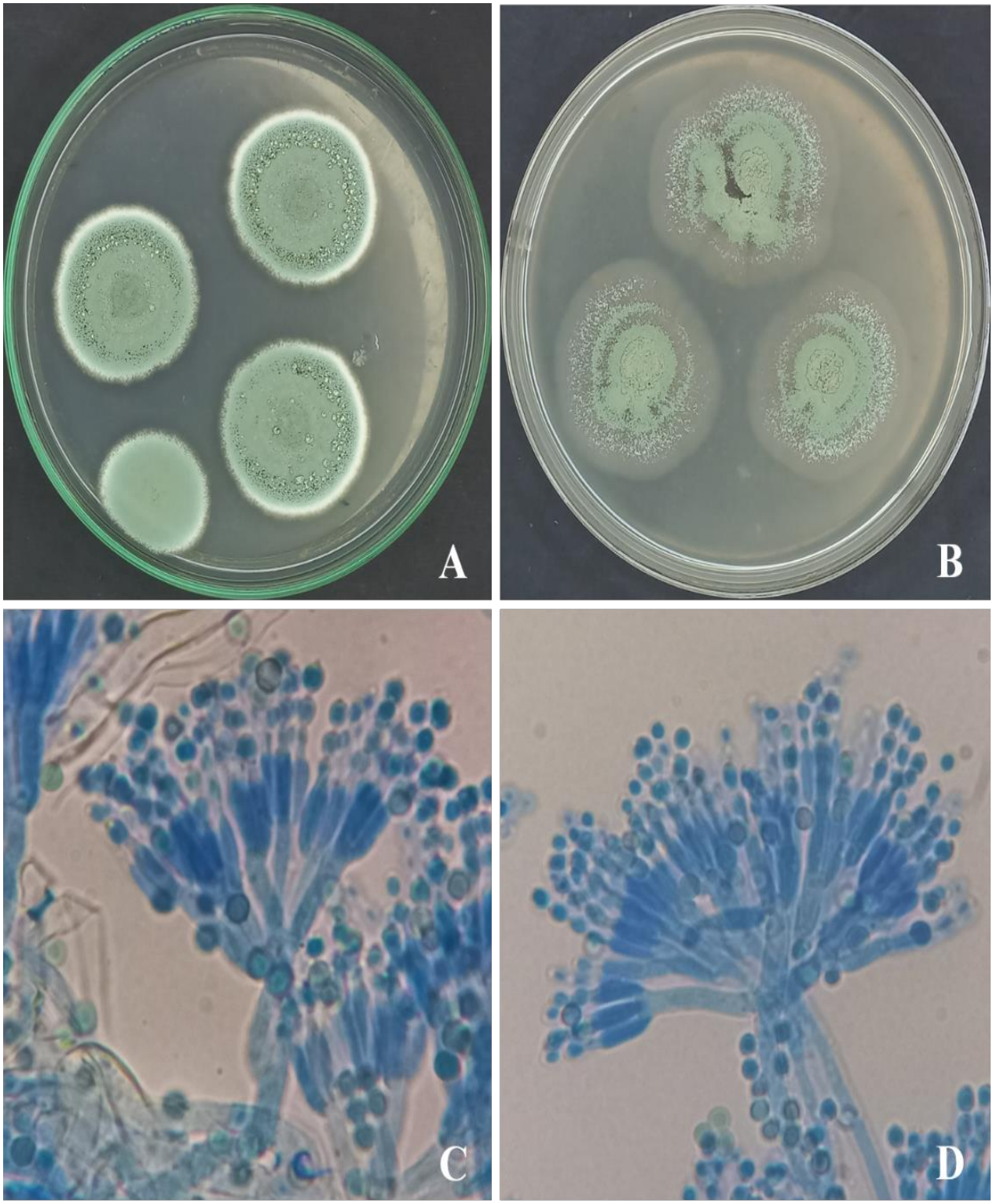

Figure27. *Penicillium crustosum*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and terverticillate penicilli.

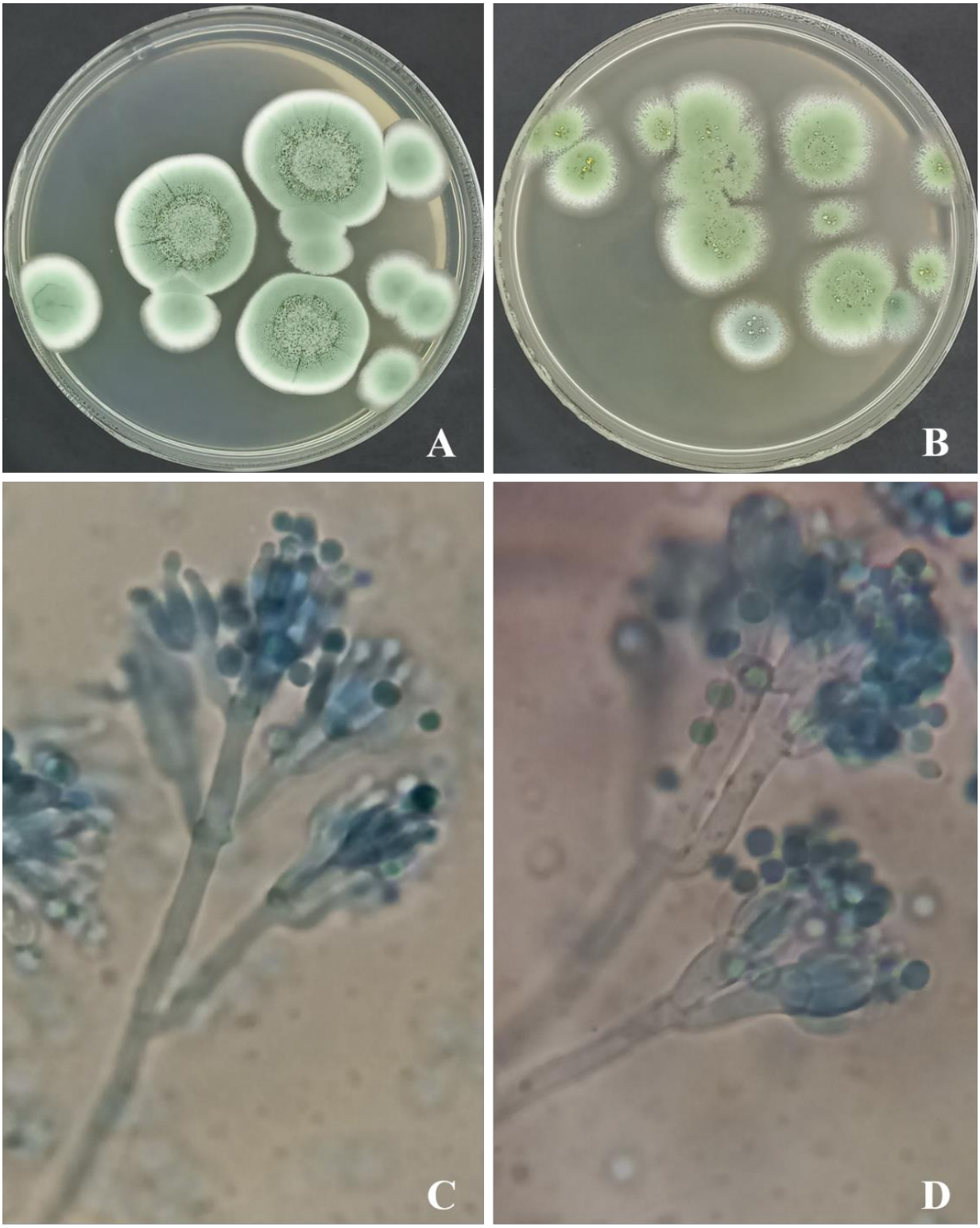

Figure28. *Penicillium expansum*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and terverticillate penicilli.

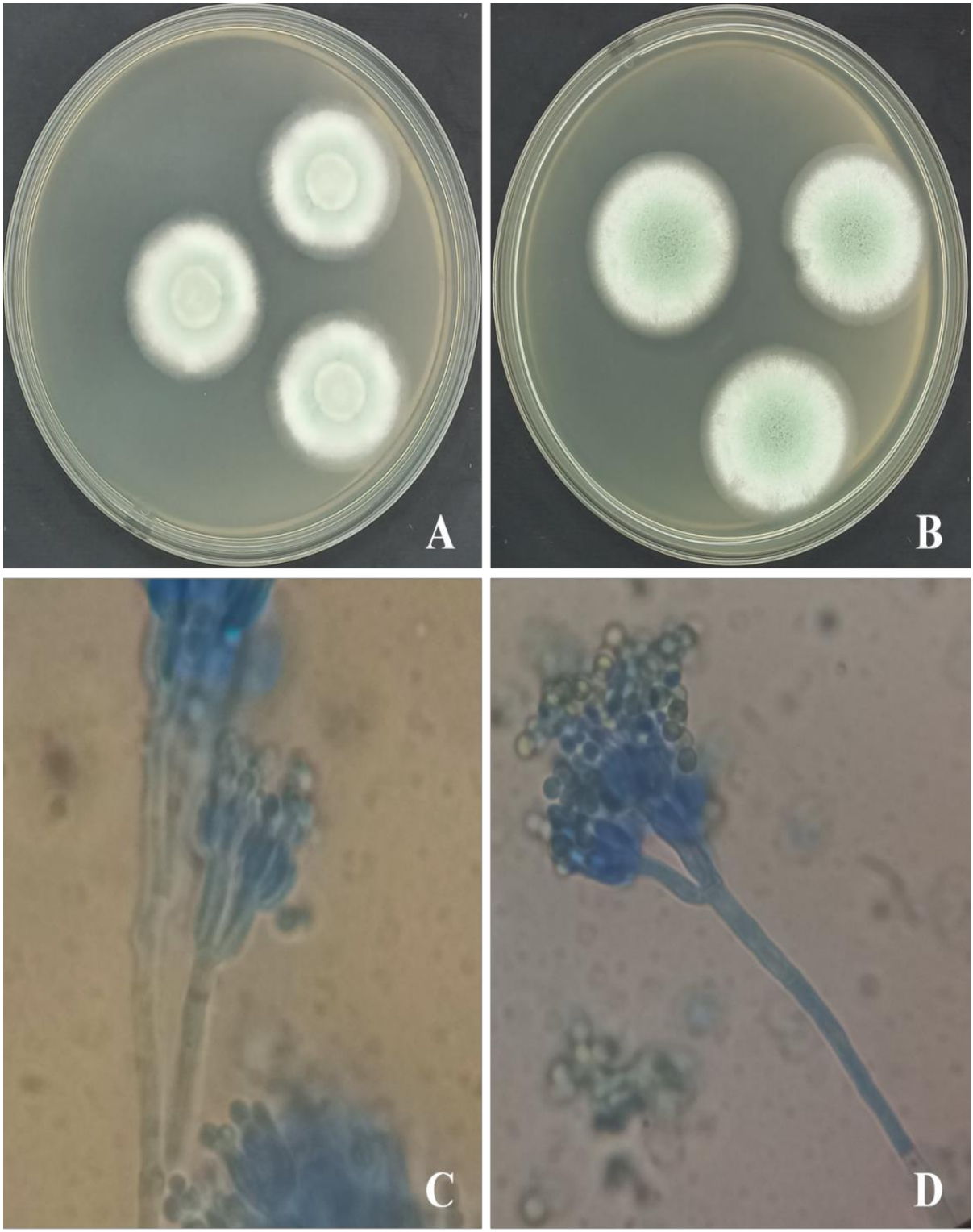

Figure29. *Penicillium griseofulvum*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and terverticillate penicilli.

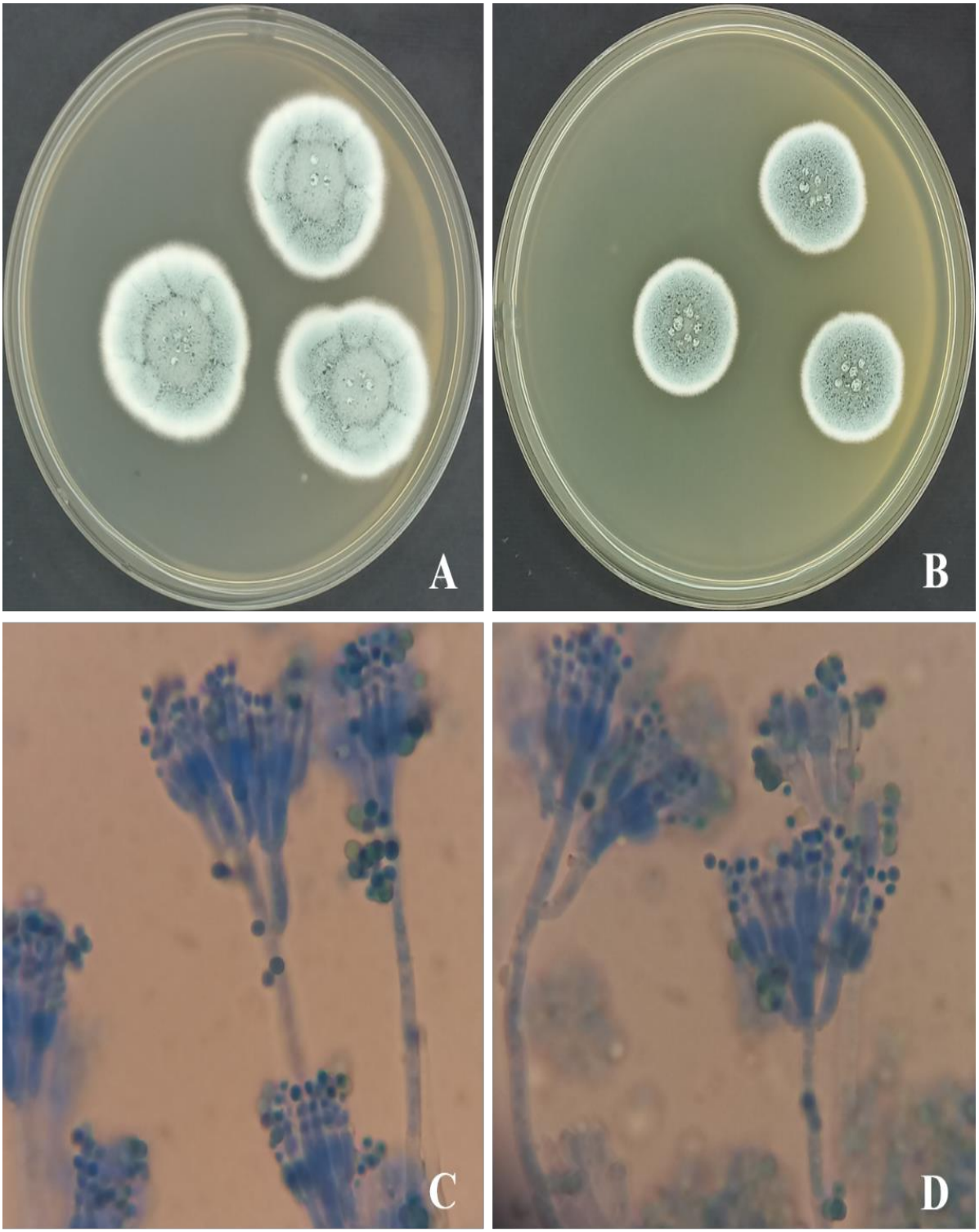

Figure30. *Penicillium puberulum*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and terverticillate penicilli.

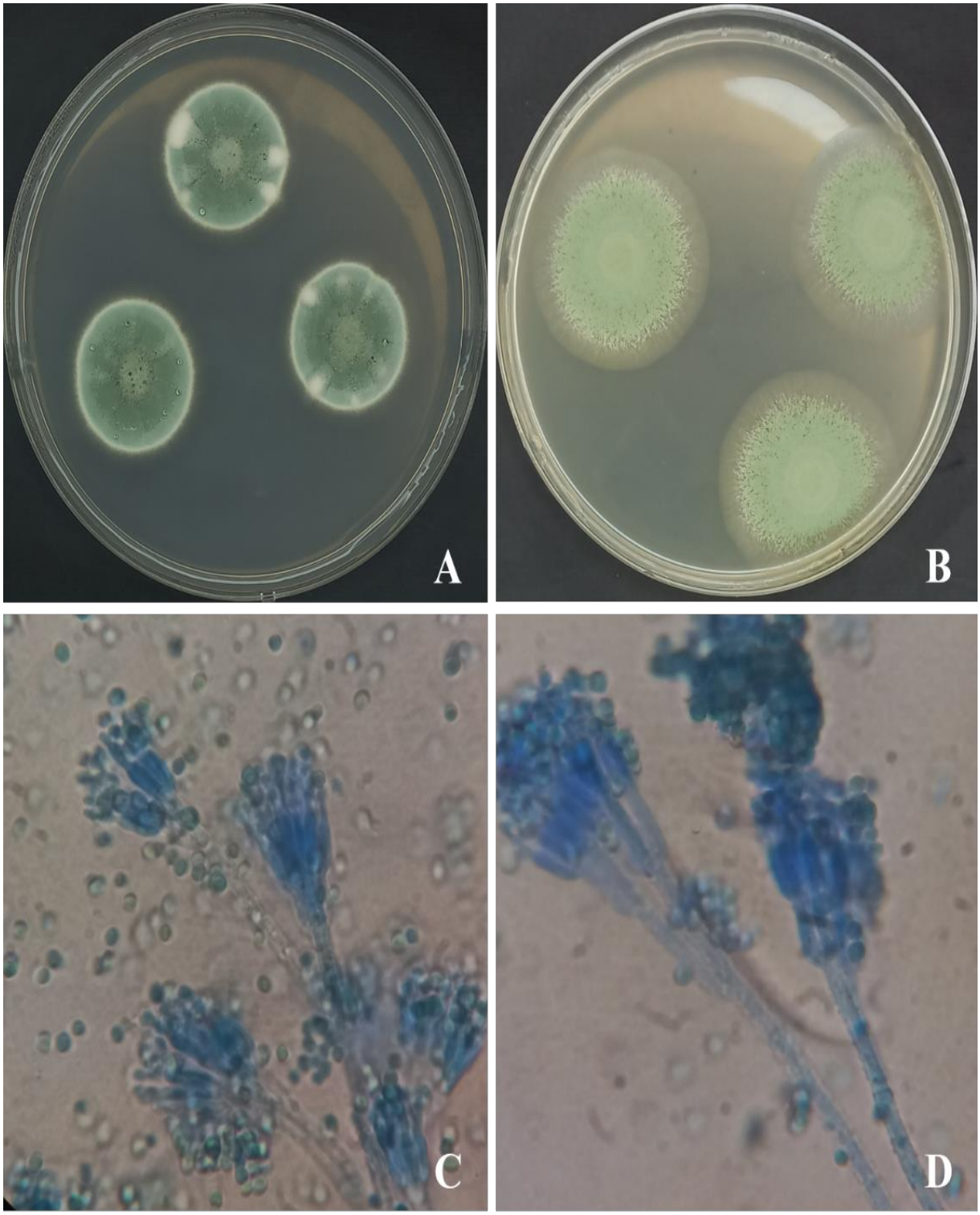

Figure31. *Penicillium roqueforti*: A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Rough conidiophores and terverticillate penicilli.

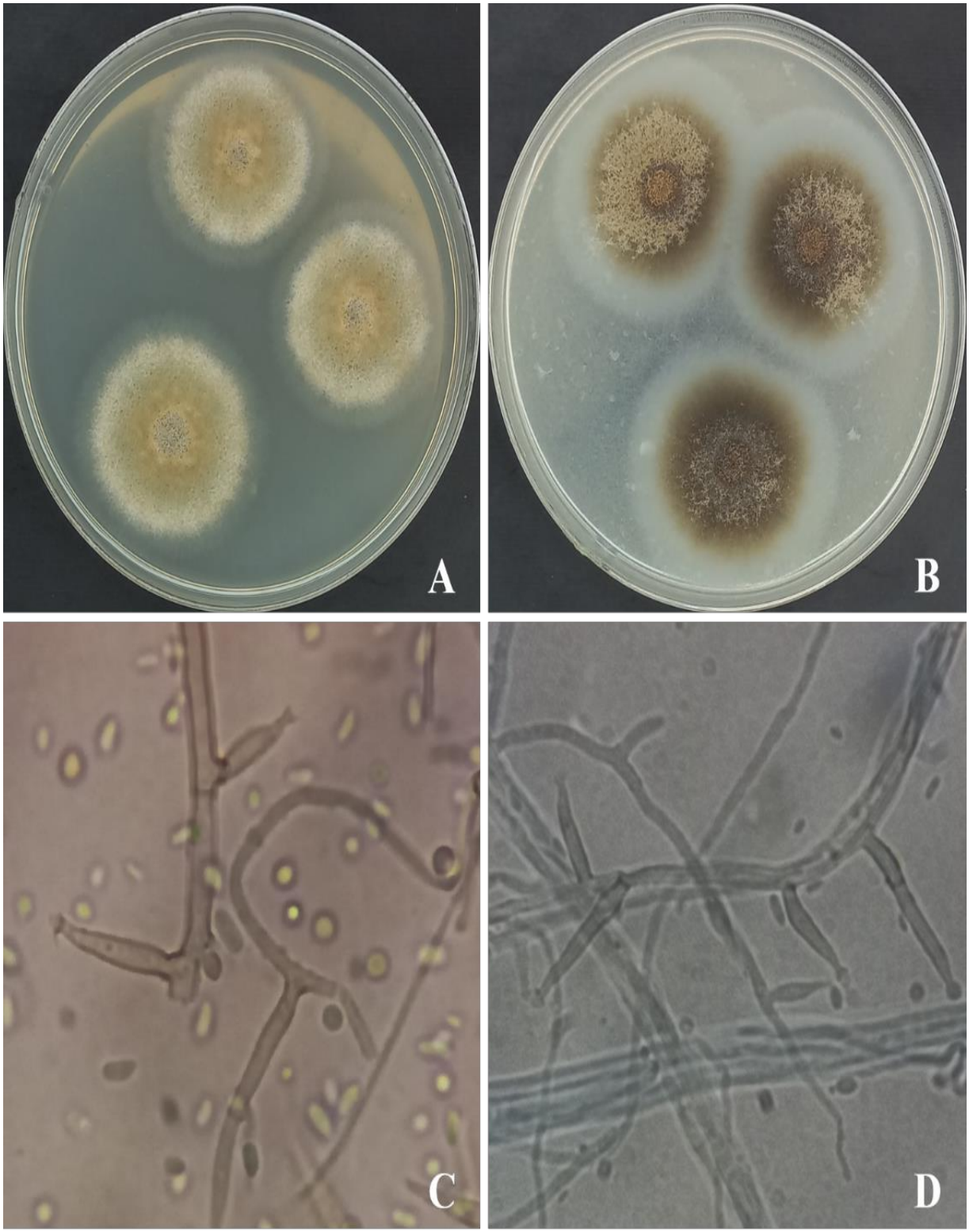

Figure 32. ***Phialophora richardisae*****:** A-B, Seven-day-old colonies on Cz and PDA at 25°C. C-D, Phialides with funnel-shaped openings and conidia.

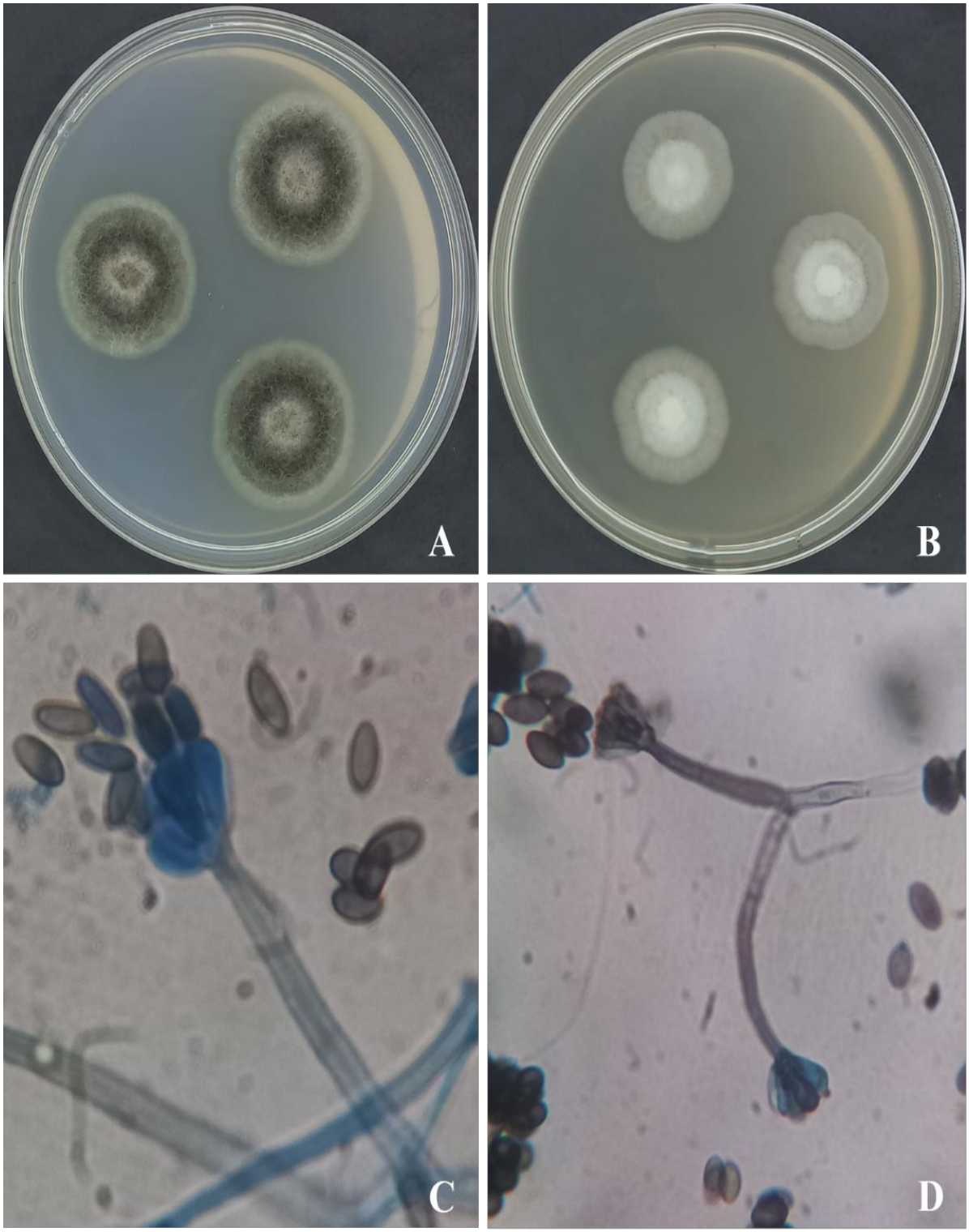

Figure33. ***Stachybotrys chartarum*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and phialides with dark, ellipsoidal conidia.

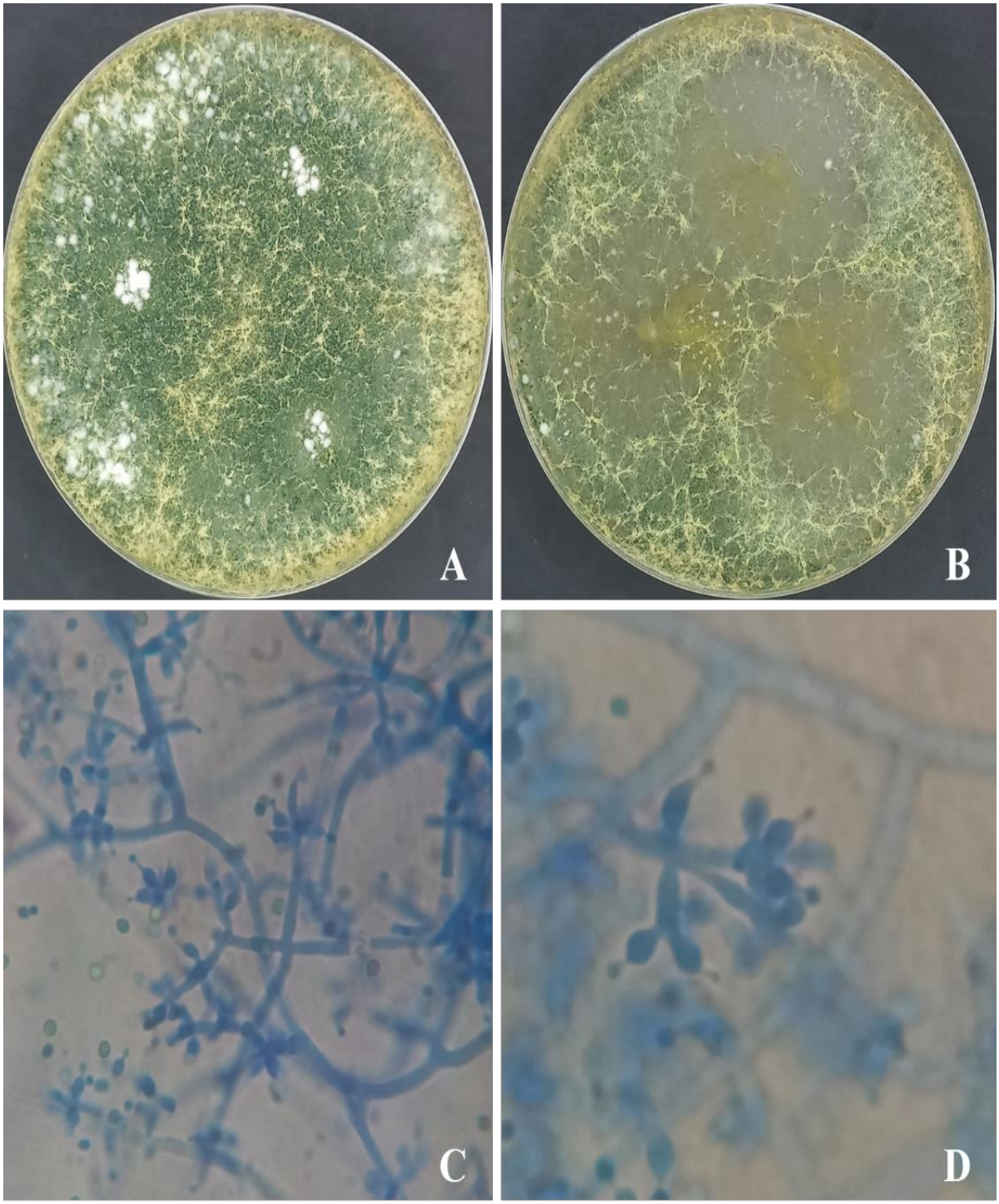

Figure34. ***Trichoderma harzianum*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and phialides.

4.2. Preliminary screening of lipolytic activity

On lipase production agar medium at two temperatures, 10 and 20°C, 102 fungal isolates from 31 species related to 12 genera were tested for their lipolytic activity. The majority of fungi could produce lipase activity at 20°C, where 98 out of 102 isolates (96% of all isolates tested), were able to do so, compared to 73 isolates (71.56% of all isolates) at 10°C. While the number of intermediate lipase producers was wider at 20°C (71) than at 10°C (29), the number of the highest lipase producers was higher at 10°C (25) than at 20°C (16). *Aspergillus* species (8) were completely positive at 20°C compared to 4 positive species at 10°C, all *Penicillium* species (9) demonstrated positive findings at both of the temperatures examined, 10 and 20°C (Table 2).

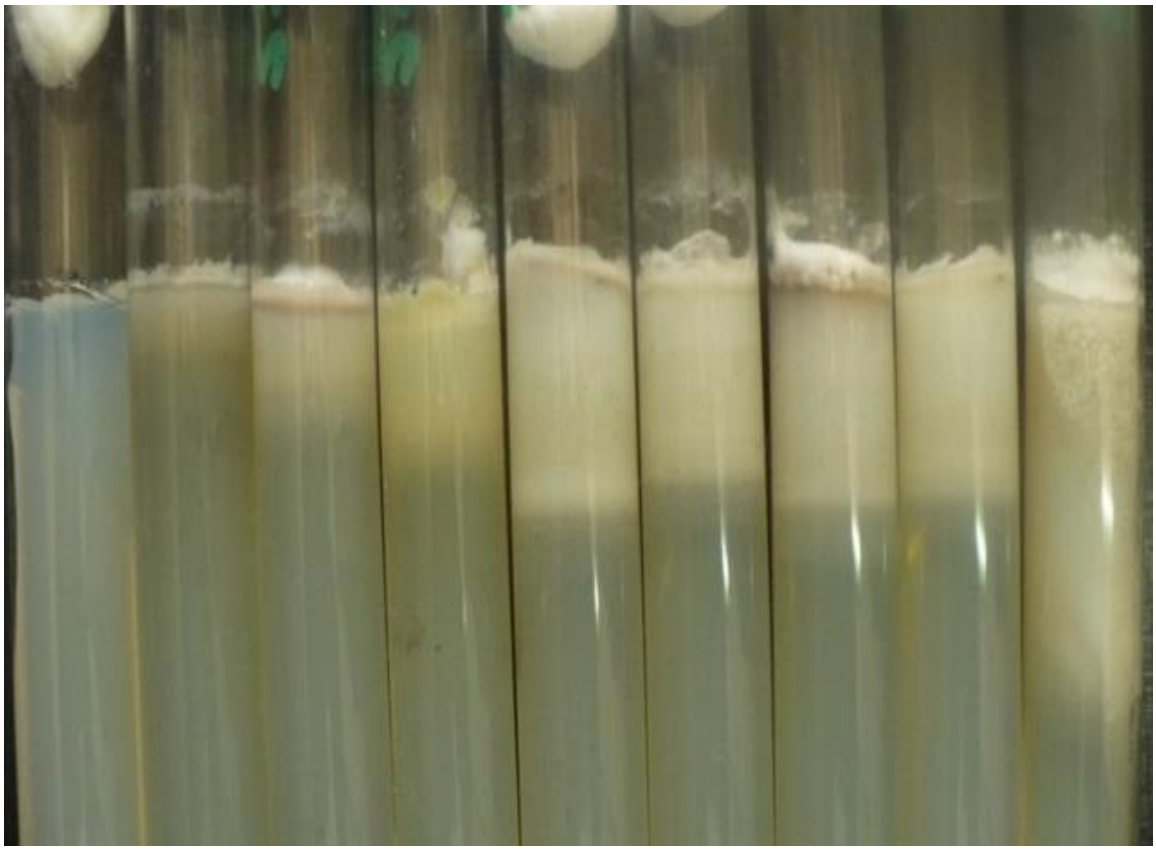

Figure 35: Preliminary screening of lipolytic activity of fungi isolated from olive oil processing wastes (Fatura).

Table 2. Preliminary screening of lipolytic activity of fungi isolated from olive oil processing wastes (Fatura) collected from different cities in Libya at 10 and 20°C on sucrose-free Cz agar supplemented with 1% tween 80.

Fungal species	No. of isolates	At 10 °C				At 20 °C			
		Positive	H	M	L	Positive	H	M	L
Alternaria	**2**	**2**	**2**			**2**		**2**	
A. alstroemeriae	1	1	1			1		1	
A. angustiovoidea	1	1	1			1		1	
Aspergillus									
A. aureolatus	6	2	1		1	5	5		
A. caespitosus	3					3	1	2	
A. deflectus	2	1			1	2		2	
A. fumigatus	1					1		1	
A. niger	4	1			1	4		3	1
A. silvaticus	1					1	1		
A. terreus	3	2			2	3		3	
A. ustus	2					2	1	1	
Fusarium									
F. oxysporum	5	5	4	1		5		5	
F. solani	19	19	13	5	1	19	3	16	
Geotrichum candidum	1	1		1		1		1	
Humicola fusco-atra	1	1			1	1			1
Microdochium nivale	1	1			1	1			1
Mucor									
M. hiemalis	7	7		7		7	1	4	2
M. racemosus	2	2			2	2		1	1
Mucor sp.	1	1		1		1		1	
Myrothecium verrucaria	1	1	1			1	1		
Penicillium									
P. aurantiogriseum	10	2	1	1		10		10	
P. brevicompactum	2	2		1	1	2		2	
P. chrysogenum	6	6		3	3	6		4	2
P. citrinum	7	6		5	1	6		6	
P. crustosum	1	1		1		1		1	
P. expansum	1	1		1		1		1	
P. griseofulvum	2	2		1	1	2		2	
P. puberulum	1	1		1		1		1	
P. roquefortii	1	1	1			1	1		
Phialophora richardisae	3					1			1
Stachybotrys chartarum	2	1			1	2			2
Trichoderma harzianum	4	4	2		2	4	2	2	
Total	**102**	**73**	**25**	**29**	**19**	**98**	**16**	**71**	**11**
No. of genera	**12**	**11**	**6**	**4**	**8**	**12**	**6**	**7**	**7**
No. of species	**31**	**26**	**9**	**13**	**14**	**31**	**9**	**23**	**8**

H= highest lipolytic activity (more than or equal 11mm), M= intermediate (from 5mm to less than 11mm), and L= low (less than 5mm).

The most active isolates were *Alternaria* (2 isolates), *Fusarium* (1), and *Penicillium* (1). Molecular identification of the most active four isolates was carried out by sequencing their internal transcribed spacer region (ITS). Pure cultures of the four fungal isolates were deposited in the culture collection of Assiut University Mycological Centre as *Alternaria* sp. AUMC 16060, *Alternaria* sp. AUMC 16062, *Fusarium* sp. AUMC 16063, and *Penicillium* sp. AUMC 16061.

4.3. Molecular identification of the potent lipase producers

4.3.1. Identification of *Alternaria* sp. AUMC 16060

For the molecular identification of *Alternaria* sp. AUMC 16060, the final ITS data collection included 18 species. The total number of characters from the ITS data set was 551, of which 478 could be correctly aligned, 30 were counted as variables, and 14 were deemed to be instructional characters. The isolate shared a branch in the evolutionary tree with *Alternaria alstroemeriae* CBS 118809 (the type strain NR_163686). *Alternaria alstroemeriae* was determined to be the species in this study, and the ITS sequence of this strain was added to GenBank as OR453765 (Figure36).

4.3.2. Identification of *Penicillium* sp. AUMC 16061

The final ITS data collection comprised 25 species for *Penicillium* sp. AUMC 16061's genetic identification. The ITS data set contained 588 characters in total, of which 488 could be successfully aligned, 58 were considered variables, and 8 were considered instructive characters. In the evolutionary tree, the isolate was associated with the type specimen, *Penicillium crustosum* FRR 1669 (NR_077153). As a result, this isolate is identified as *Penicillium crustosum*, and the strain's ITS sequence has been entered into the GenBank database under the accession number OR453768 (Figure 37).

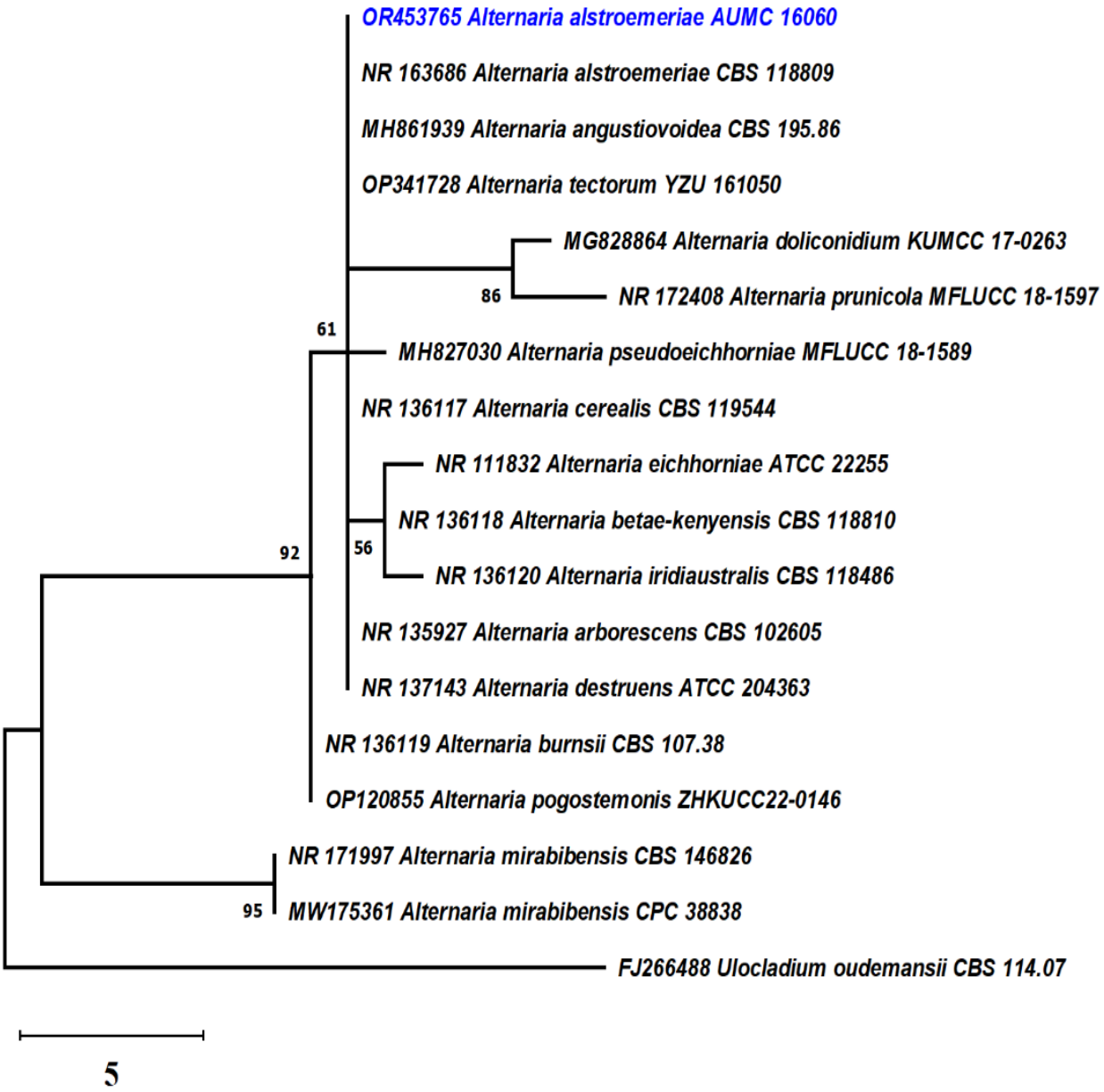

Figure 36. The most parsimonious tree obtained from a heuristic search (1000 replications) of *Alternaria alstroemeriae* AUMC 16060's ITS sequence (in blue color) compared to other closely similar ITS sequences belonging to *Alternaria* in GenBank. Bootstrap support values are indicated near the respective nodes. The tree is rooted to *Ulocladium oudemansii* CBS 114.07 as out group.

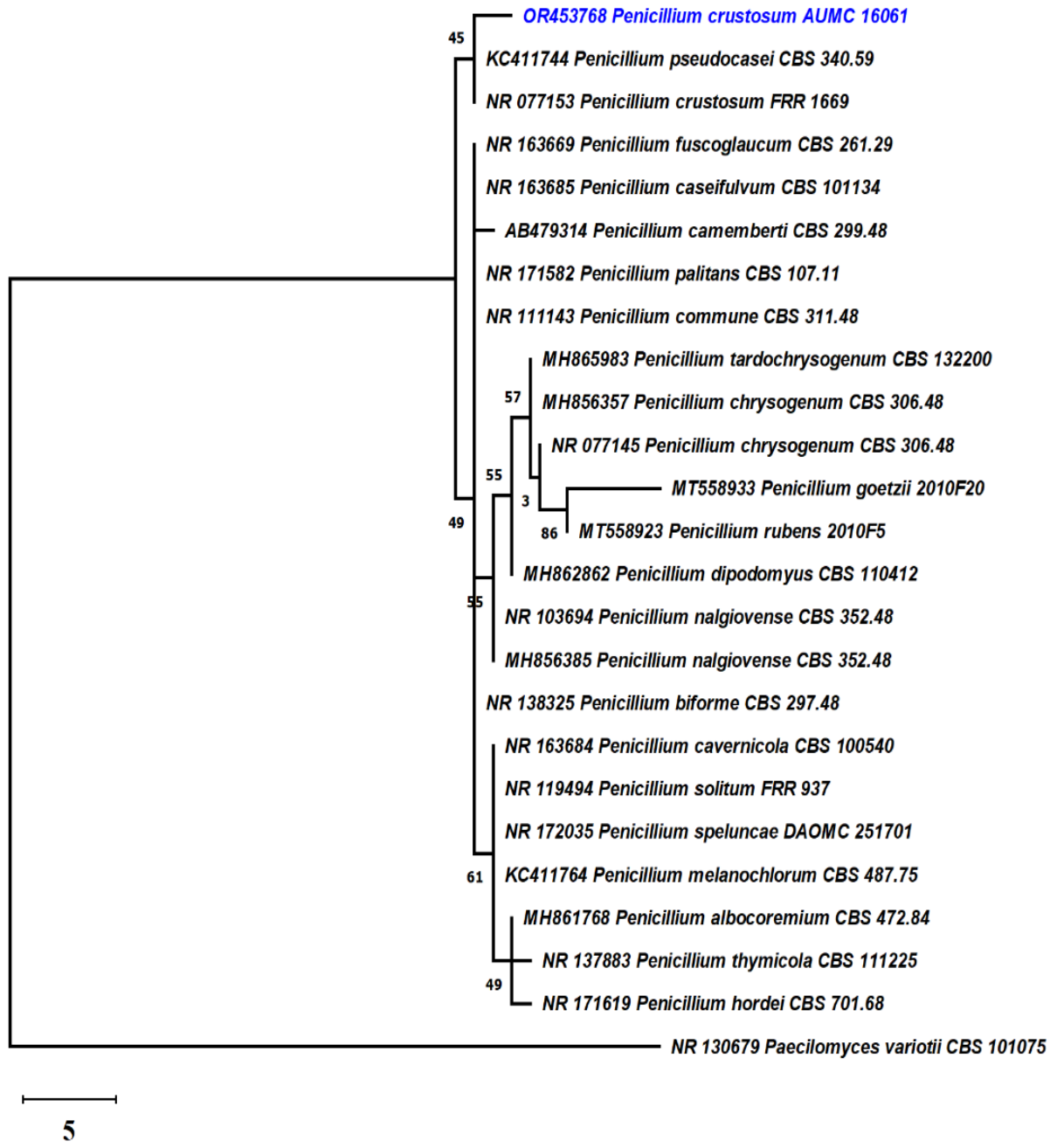

Figure 37. The most parsimonious tree obtained from a heuristic search (1000 replications) of *Penicillium crustosum* AUMC 16061's ITS sequence (in blue color) compared to other closely similar ITS sequences belonging to genus *Penicillium* in GenBank. Bootstrap support values are indicated near the respective nodes. The tree is rooted to *Paecilomyces variotii* CBS 101075 as out group.

4.3.3. Identification of *Alternaria* sp. AUMC 16062

The final ITS data collection comprised 17 species for the genetic identification of *Alternaria* sp. AUMC 16062. The ITS data set contained a total of 550 characters, of which 493 could be successfully aligned, 25 were counted as variables, and 2 were counted as instructive characters. In the evolutionary tree, the isolate belonged to the same clade as *Alternaria angustiovoidea* KH35 (OM202461). This isolate is identified to be *Alternaria angustiovoidea*, and the ITS sequence of this strain was uploaded to the GenBank database as OR453769 (Figure 38).

4.3.4. Identification of *Fusarium* sp. AUMC 16063

The final ITS data collection comprised 19 species for the genetic identification of *Fusarium* sp. AUMC 16063. The ITS data set contained a total of 607 characters, of which 475 could be successfully aligned, 134 were counted as variables, and 33 were counted as instructive characters. In the evolutionary tree, the isolate belonged to the same clade as *Fusarium solani* CBS 127319 (MH864522), *F. solani* CBS 140079 (NR_163531, type species), and *F. solani* CBS 208.29 (MH855046). As a result, this isolate is identifiedas *Fusarium solani*, and the ITS sequence of this strain was uploaded to the GenBank database as OQ878686 (Figure 39).

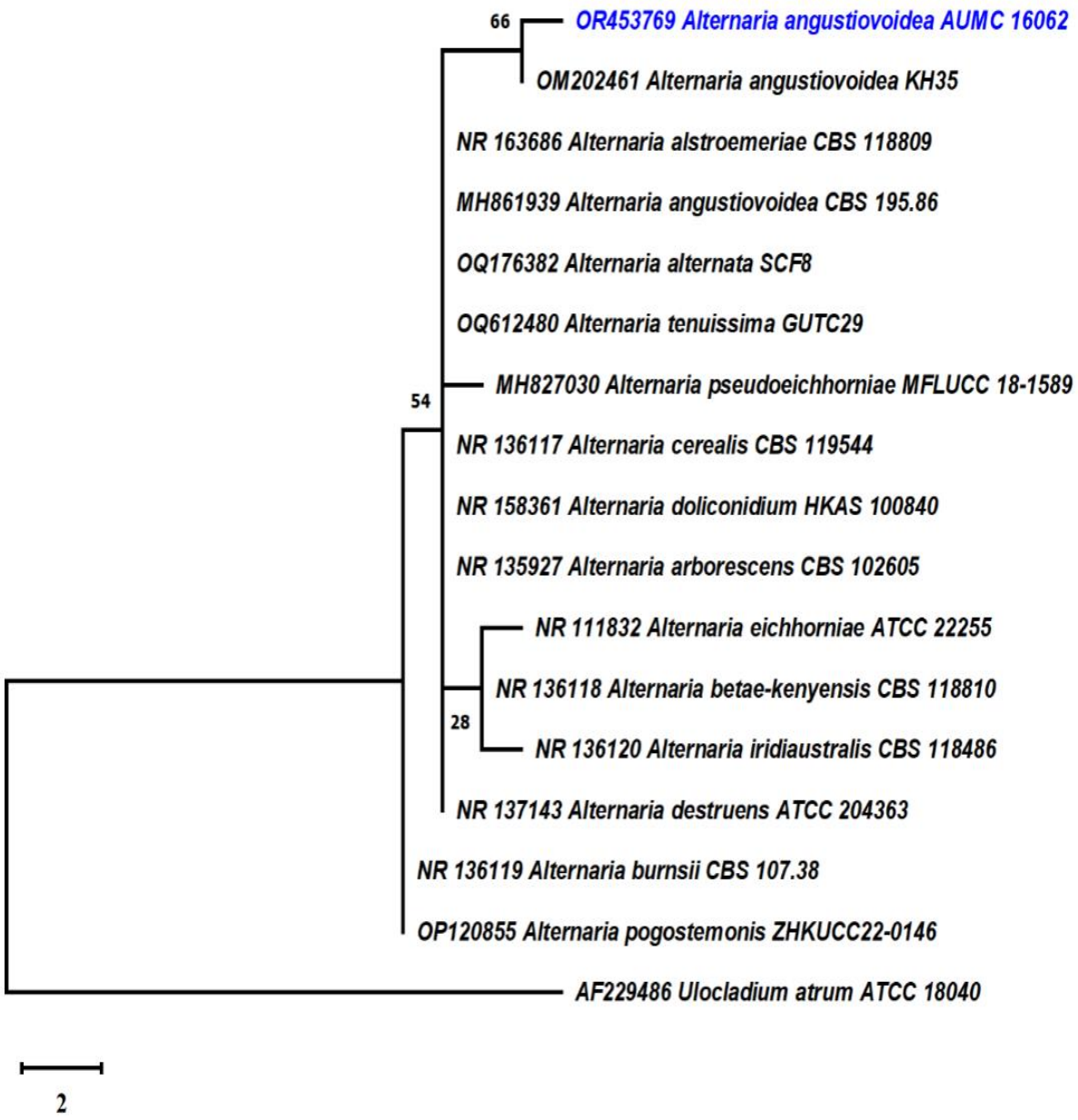

Figure 38. The most parsimonious tree obtained from a heuristic search (1000 replications) of *Alternaria angustiovoidea* AUMC 16062's ITS sequence (in blue color) compared to other closely similar ITS sequences belonging to *Alternaria* in GenBank. Bootstrap support values are indicated near the respective nodes. The tree is rooted to *Ulocladium atrum* ATCC 18040 as out group.

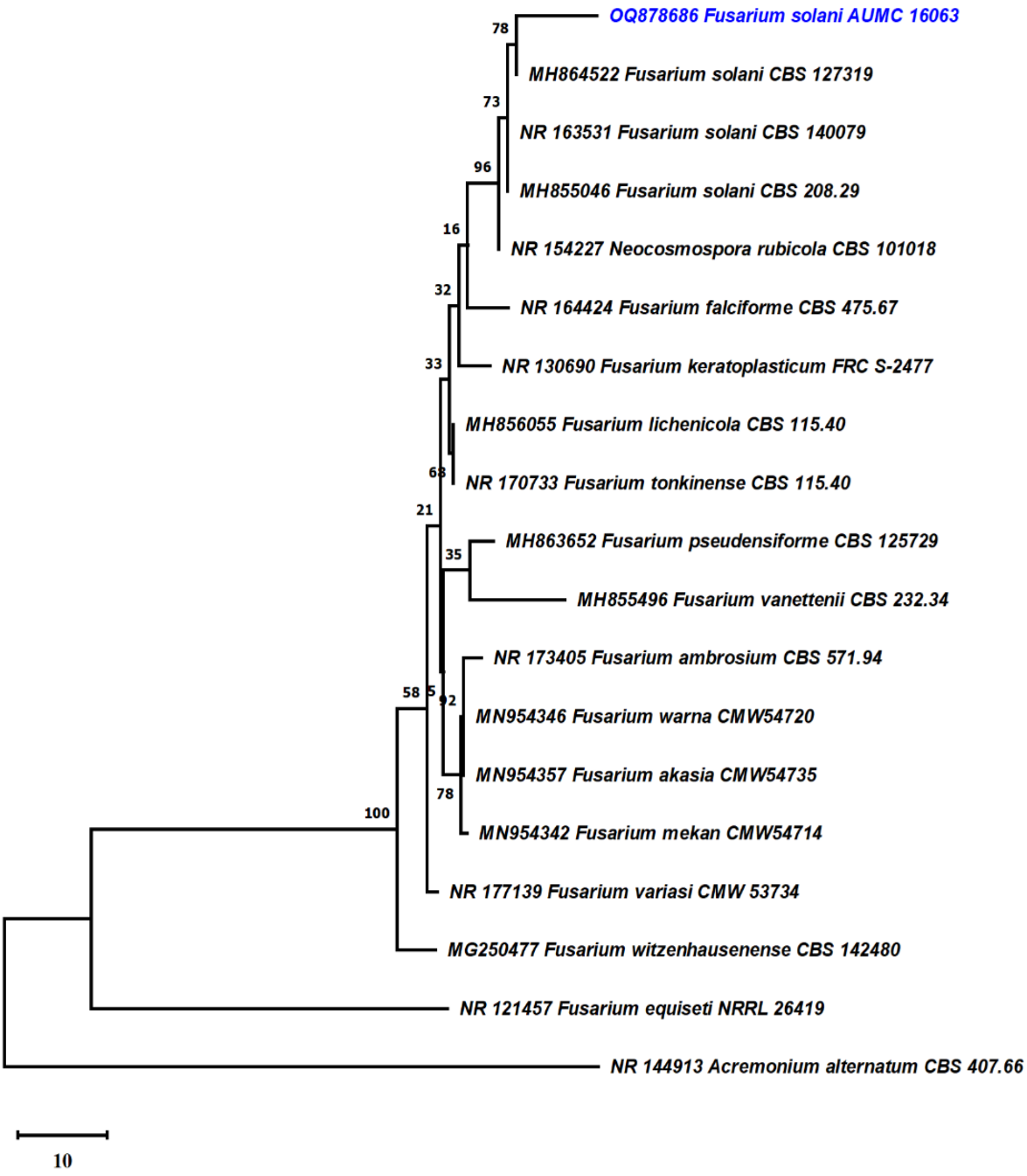

Figure 39. The most parsimonious tree obtained from a heuristic search (1000 replications) of*Fusarium solani* AUMC 16063's ITS sequence (in blue color) compared to other closely similar ITS sequences belonging to *Fusarium* in GenBank. Bootstrap support values are indicated near the respective nodes. The tree is rooted to *Acremonium alternatum* CBS 407.66 as out group.

4.4. Effect of medium's pH and incubation temperature on lipase production

- At 10°C

The four powerful fungal strains' production of cold-active lipase was maximized by optimizing some nutritional and environmental factors. For *Alternaria alstroemeriae*, *A. angustiovoidea*, and *Fusarium solani*, pH 3 produced cold-active lipase enzyme activity of 32.66, 31.5, and 33.83 U/mL/min, respectively, at 10°C. *Penicillium crustosum* performed best at pH 5, yielding 9.91 U/mL/min. The most potent strain was *Fusarium solani* when compared to the others. It generated the highest levels of specific activity (512.5 U/mg protein) and cold-active lipase activity (33.83 U/mL/min) (Table 3; Figures 40–43).

Table 3. Effect of medium's pH on the activity of lipase produced by the highest producing fungi at 10°C

pH	*A. alstroemeriae* AUMC 16060		*A. angustiovoidea* AUMC 16062		*F. solani* AUMC 16063		*P.crustosum* AUMC 16061	
	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein
3	32.66±4^{a}	269.9±36.36^{a}	31.5±1.6^{a}	85.36±4.33^{a}	33.83±1.4^{a}	512.5±21.2^{a}	4.7±0.82^{c}	18.50±3.23^{e}
4	24.5±1.2^{b}	81.21±4^{b}	26.24±1.8^{b}	83.5±5.73^{b}	23.33±1.1^{b}	85.45±4^{c}	4.7±0.67^{c}	45.19±6.44^{c}
5	12.24±1.5^{c}	57.5±7^{c}	13.10±1.5^{c}	57.45±6.58^{c}	4.1±0.6d	25±3.66^{f}	9.91±0.73^{a}	86.92±6.4^{a}
6	4.08±0.8^{f}	13.03±2.55^{e}	6.41±0.8^{d}	21.01±2.66^{e}	0^{f}	0^{g}	2.33±0.5^{d}	14.29±3^{f}
7	6.41±0.5^{d}	24.3±1.9^{d}	6.10±0.76^{d}	28.24±3.52^{d}	5.24±0.42^{c}	93.57±7.5^{b}	8.74±1^{b}	53.29±9.52^{b}
8	0^{g}	0^{g}	0^{f}	0^{h}	0^{f}	0^{g}	5.24±0.92^{c}	22.88±4^{d}
9	2.91±0.3^{f}	25.1±2.58^{d}	1.74±0.2^{e}	14.26±1.64^{f}	4.1±0.5^{d}	53.94±6.58^{d}	5.24±0.71^{c}	11.54±1.56^{g}
10	0.583±0.2^{g}	4.3±1.47^{f}	0.58±0.1^{f}	4.83±0.83^{g}	2.33±0.2^{e}	38.19±3.27^{e}	2.91±0.3^{d}	4.58±0.47^{h}

Figures in Table are mean of three replicates (n=3)±SD. At the 0.05 level of probability,means in a column with the same letters are not statistically different.

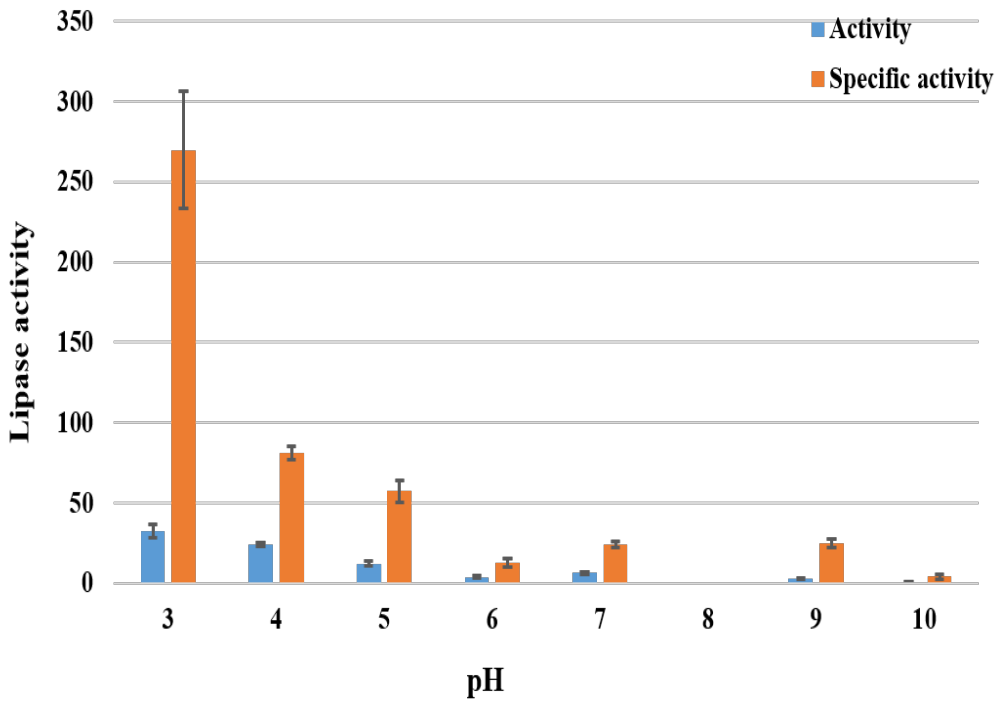

Figure40. Effect of medium's pH on the activity of cold-active lipase produced by *A. alstroemeriae* AUMC 16060 at 10 °C.

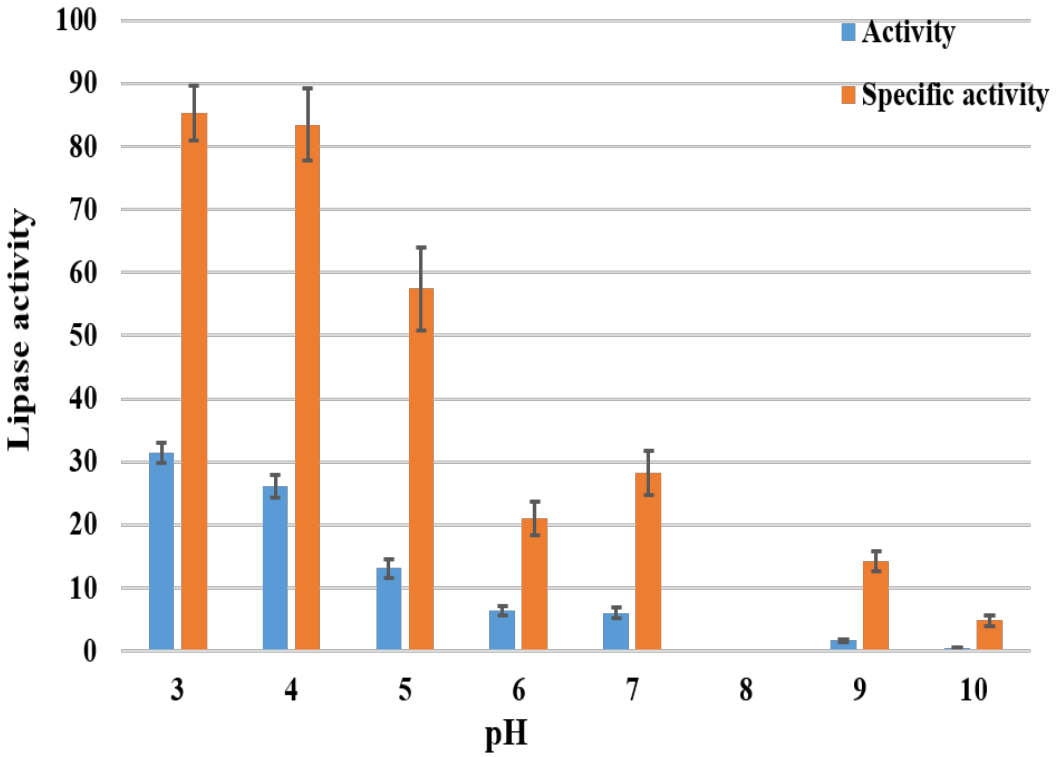

Figure41. Effect of medium's pH on the activity of cold-active lipase produced by *A. angustiovoidea* AUMC 16062 at 10 °C.

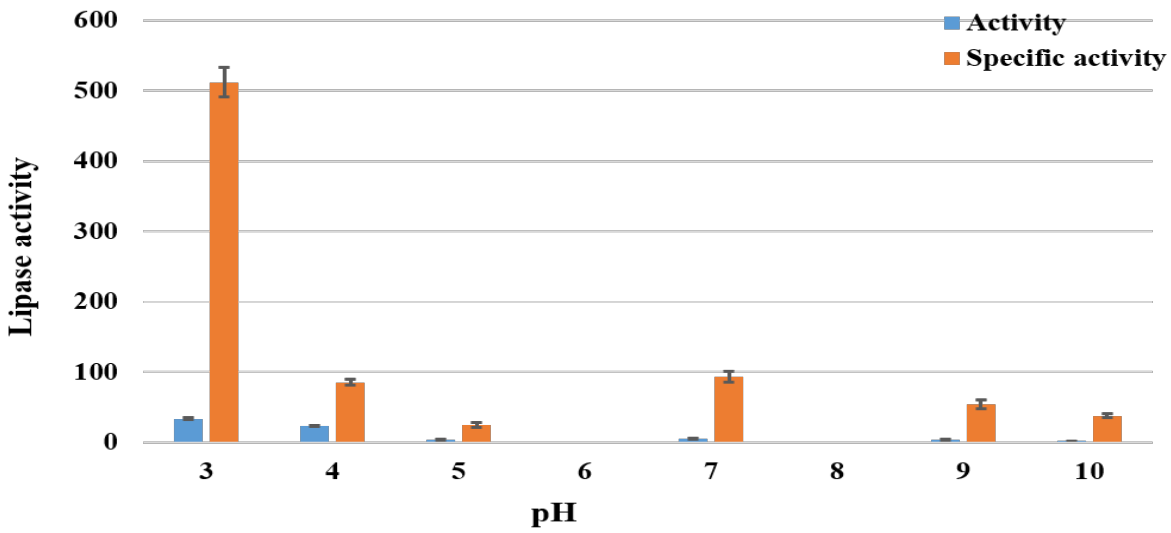

Figure42. Effect of medium's pH on the activity of cold-active lipase produced by *F. solani* AUMC 16063 at 10 °C.

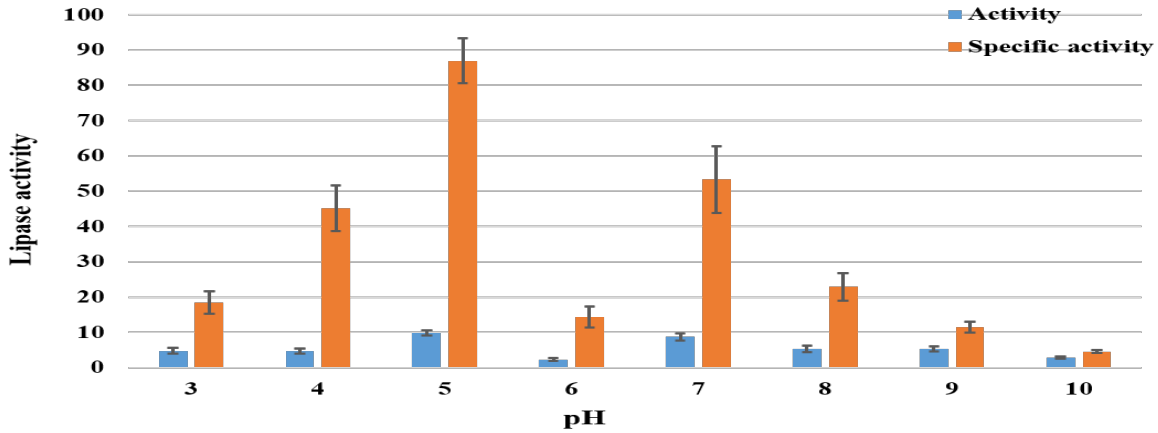

Figure43. Effect of medium's pH on the activity of cold-active lipase produced by *P. crustosum* AUMC 16061 at 10 °C.

- At 15°C

At 15°C, pH 3 produced the best cold-active lipase enzyme activity for *Alternaria alstroemeriae*, *A. angustiovoidea*, and *Fusarium solani*, with values of 33.83, 39.66, and 45.49 U/mL/min, respectively. At pH 7, *Penicillium crustosum* produced 10.49 U/mL/min, which was its best output. Comparing the tested strains, *Fusarium solani* was the most powerful. It produced the highest levels of both cold-active lipase activity (45.49 U/mL/min) and specific activity (385.5 U/mg protein) (Table 4; Figures 44–47).

Table 4. Effect of pH on the activity of lipase produced by the highest producing fungi at 15°C.

pH	*A. alstroemeriae* AUMC 16060		*A. angustiovoidea* AUMC 16062		*F. solani* AUMC 16063		*P.crustosum* AUMC 16061	
	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein
3	33.83±3.2^{a}	70.04±6.62^{b}	39.66±4.2^{a}	60.73±6.43^{b}	45.49±4.5^{a}	385.5±38.13^{a}	4.08±0.5^{c}	9.27±1.13^{c}
4	18.66±1.2^{b}	97.69±6.28^{a}	26.83±2.8^{b}	53.76±5.6^{c}	11.08±0.6^{b}	85.89±4.65^{b}	4.08±0.6^{c}	17.14±2.52^{b}
5	11.08±0.84^{c}	43.28±3.28^{c}	25.08±2.2^{c}	87.38±7.66^{a}	4.66±0.3^{e}	23.77±1.53^{d}	2.33±0.8^{d}	7.61±2.66^{b}
6	9.91±0.65^{c}	16.08±1.055^{e}	13.41±1.2^{d}	20.03±1.79^{d}	3.49±0.11^{f}	15.37±0.484^{g}	0^{e}	0^{d}
7	10.49±1^{c}	17.02±1.62^{e}	12.83±0.8^{d}	19.95±1.24^{d}	5.83±0.2^{d}	26.99±0.926^{c}	10.49±1.2^{a}	23.36±2.67^{a}
8	11.08±1.1^{c}	18.62±1.85^{d}	8.16±0.6^{e}	10.96±0.8^{f}	4.66±0.2^{e}	21.98±0.94^{e}	5.83±0.7^{b}	16.75±2^{b}
9	5.24±0.5^{d}	10.52±1^{f}	8.16±0.64^{e}	13.97±1.1^{e}	5.24±0.3d^{e}	17.29±1^{f}	5.83±0.62^{b}	8.95±0.95^{c}
10	11.08±0.83^{c}	17.58±1.3^{de}	5.24±0.46^{f}	7.47±0.66^{g}	7.58±0.64^{c}	17.62±1.48^{f}	4.66±0.32b^{c}	8.07±0.55^{c}

Figures in Table are mean of three replicates (n=3)±SD. At the 0.05 level of probability,means in a column with the same letters are not statistically different.

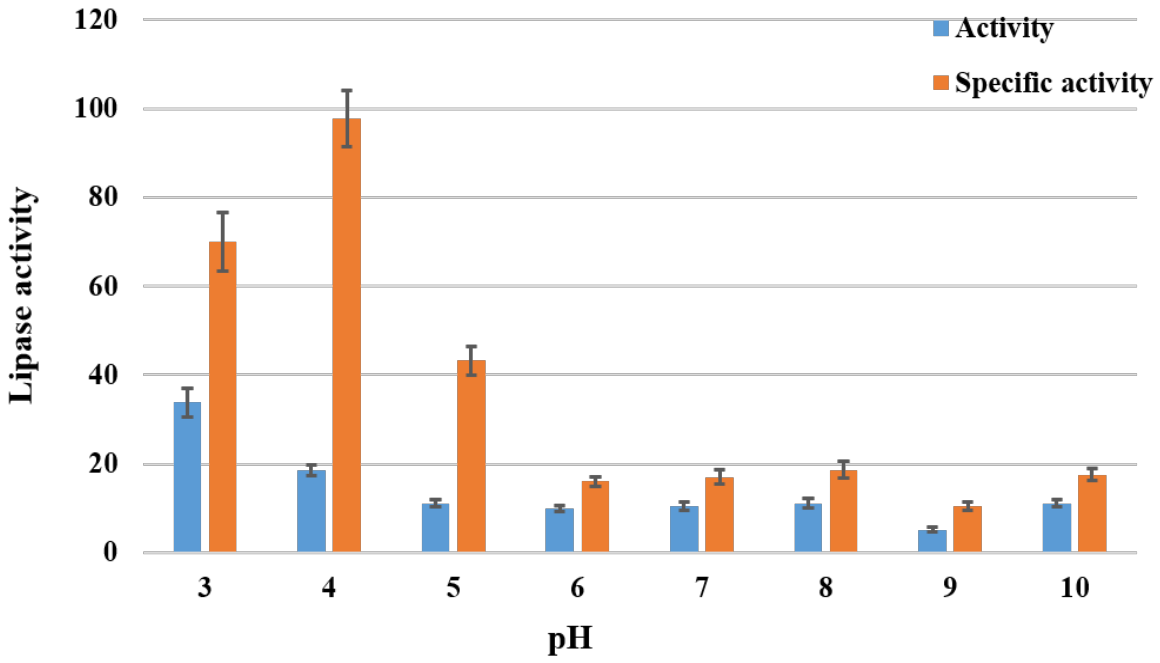

Figure44. Effect of medium's pH on the activity of cold-active lipase produced by *A. alstroemeriae* AUMC 16060 at 15°C.

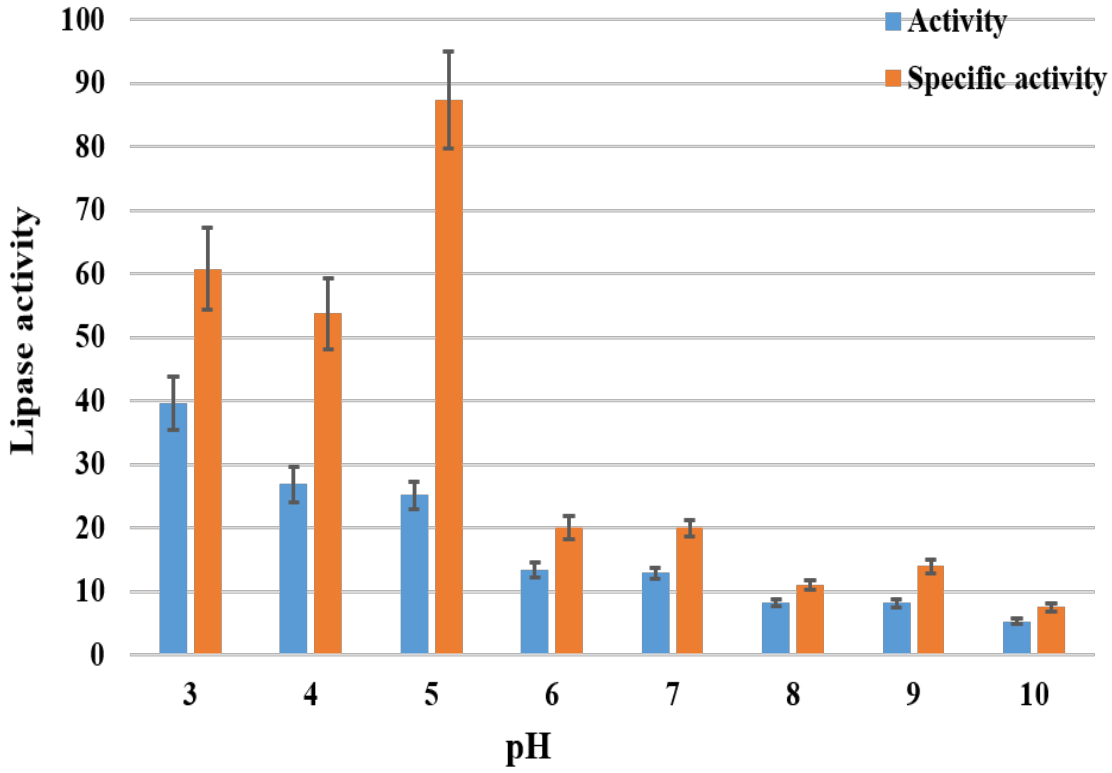

Figure45. Effect of medium's pH on the activity of cold-active lipase produced by *A. angustiovoidea* AUMC 16062 at 15°C.

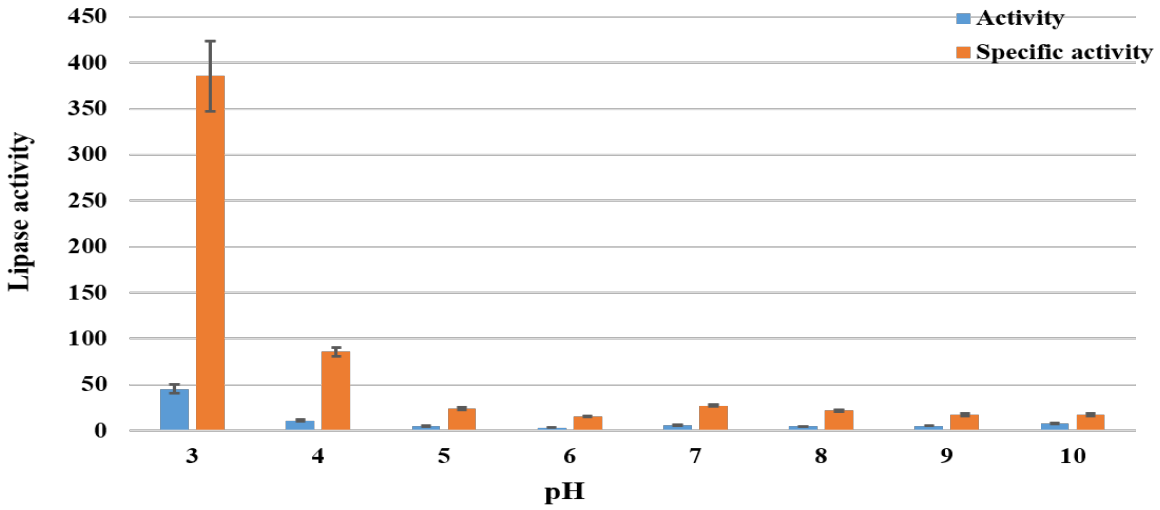

Figure46. Effect of medium's pH on the activity of cold-active lipase produced by *F. solani* AUMC 16063 at 15°C.

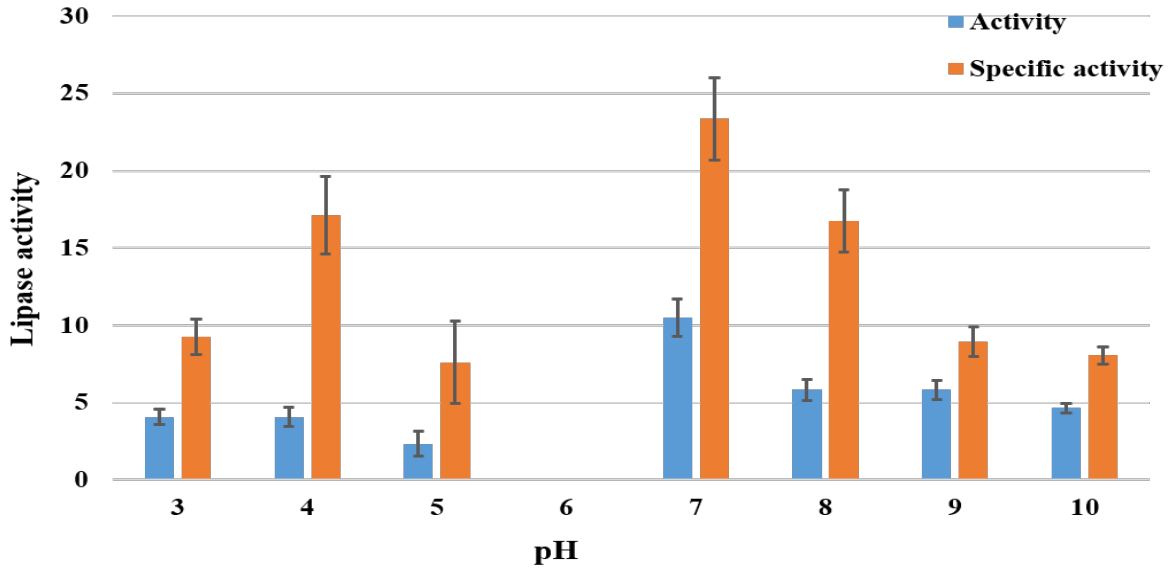

Figure47. Effect of medium's pH on the activity of cold-active lipase produced by *P. crustosum* AUMC 16061 at 15°C

- At 20°C

At pH 3.0, *Alternaria alstroemeriae* and *Fusarium solani* exhibited their highest levels of cold-active lipase activity (34.41 and 36.74 U/mL/min, respectively) and specific activity (296.63 and 854.4 U/mg protein, respectively). The cold-active lipase activity (22.16 U/mL/min) and specific activity (107.05 U/mg protein) of *Alternaria angustiovoidea* were at their highest levels at pH 4, respectively. The maximum cold-active lipase activity was, however, produced by *Penicillium crustosum* at pH 7.0, with 33.24 U/mL/min and 170.46 U/mL/min, respectively (Table 5; Figures 48-51).

Table 5. Effect of pH on the activity of lipase produced by the highest producingfungi at 20 °C

pH	*A. alstroemeriae* AUMC 16060		*A. angustiovoidea* AUMC 16062		*F. solani* AUMC 16063		*P.crustosum* AUMC 16061	
	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein	Activity U/mL/min	Specific activity U/mg protein
3	34.41±3.5^{a}	296.63±30.17^{a}	9.91±1.1^{c}	42.33±4.7^{d}	36.74±4^{a}	854.4±93^{a}	2.33±0.4^{de}	9.58±1.64^{f}
4	6.41±0.52^{c}	31.42±2.6^{c}	22.16±3.6^{a}	107.05±18^{a}	1.74±0.5^{f}	14.5±4.16^{g}	3.49±0.3^{d}	19.94±1.7^{e}
5	7.58±0.86^{c}	53.75±6.1^{b}	16.33±2^{b}	101.42±12.42^{b}	6.99±0.85^{cd}	69.20±8.5^{d}	1.74±0.2^{e}	9.61±1.1^{f}
6	12.24±1.2^{b}	53.21±5.2^{b}	1.74±0.8^{g}	7.63±3.5^{g}	4.66±0.32^{e}	51.77±3.55^{e}	6.99±0.8^{bc}	43.14±4.94^{d}
7	7.58±1^{c}	32.81±4.33^{c}	7.58±0.76^{d}	78.95±7.9^{c}	8.16±1^{c}	83.26±10.2^{b}	33.24±4.6^{a}	170.46±23.6^{a}
8	4.66±0.5^{d}	21.98±2.36^{d}	4.66±0.24^{e}	9.15±0.48^{g}	2.91±0.22^{f}	18.30±1.38^{f}	8.16±1^{b}	48±5.88^{b}
9	6.99±0.8^{c}	18.29±2.1^{e}	4.66±0.2^{e}	27.57±1.18^{e}	14.58±1.4^{b}	72.9±7^{c}	8.16±0.9^{b}	43.17±4.76^{cd}
10	2.33±0.3^{e}	14.74±1.9^{f}	3.49±0.3^{f}	21.41±1.84^{f}	5.83±0.92^{de}	11.18±1.77^{h}	6.41±0.75^{c}	44.51±5.2^{c}

Figures in Table are mean of three replicates (n=3)±SD. At the 0.05 level of probability,means in a column with the same letters are not statistically different

\

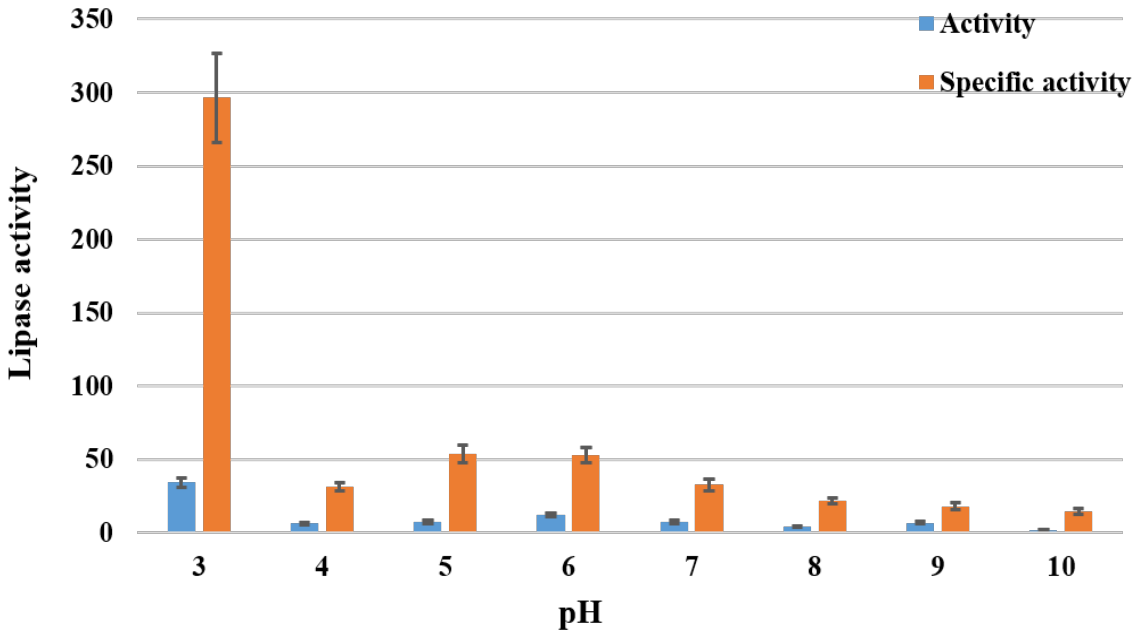

Figure48. Effect of medium's pH on the activity of cold-active lipase produced by *A. alstroemeriae* AUMC 16060 at 20°C.

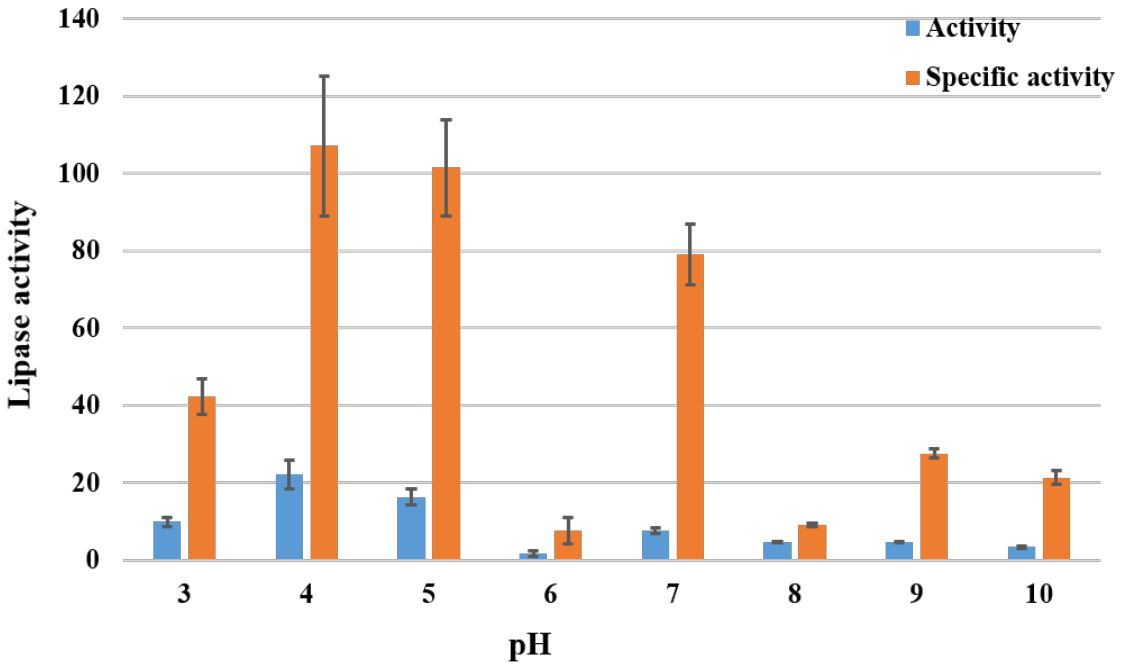

Figure49. Effect of medium's pH on the activity of cold-active lipase produced by *A. angustiovoidea* AUMC 16062 at 20°C.

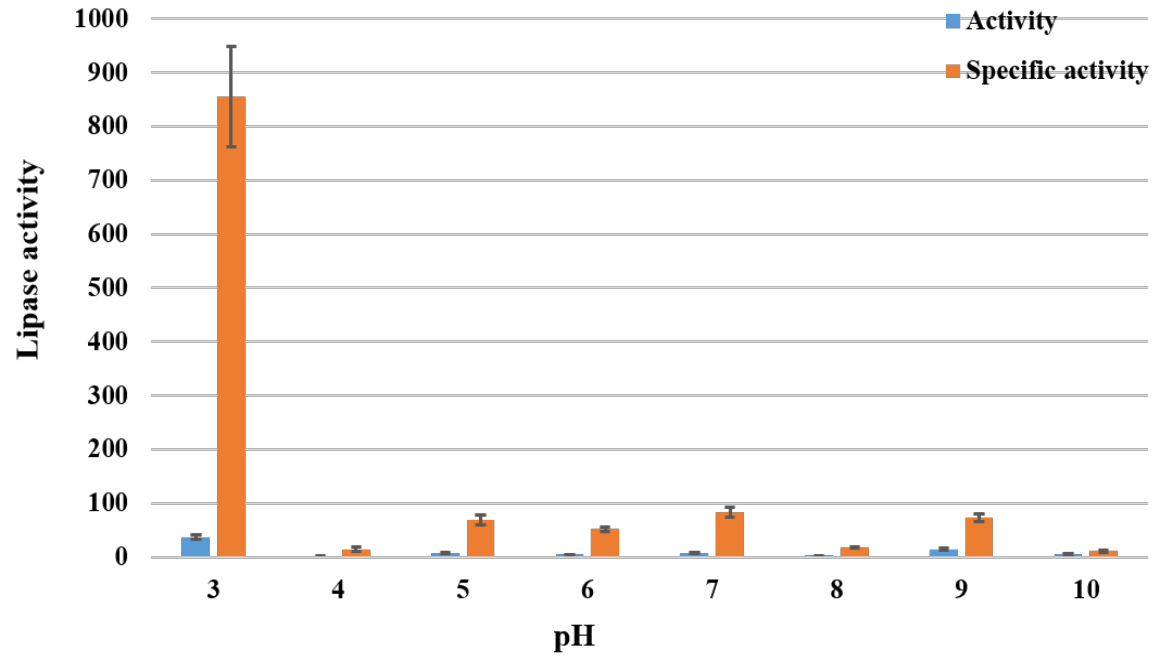

Figure50. Effect of medium's pH on the activity of cold-active lipase produced by *F. solani* AUMC 16063 at 20°C.

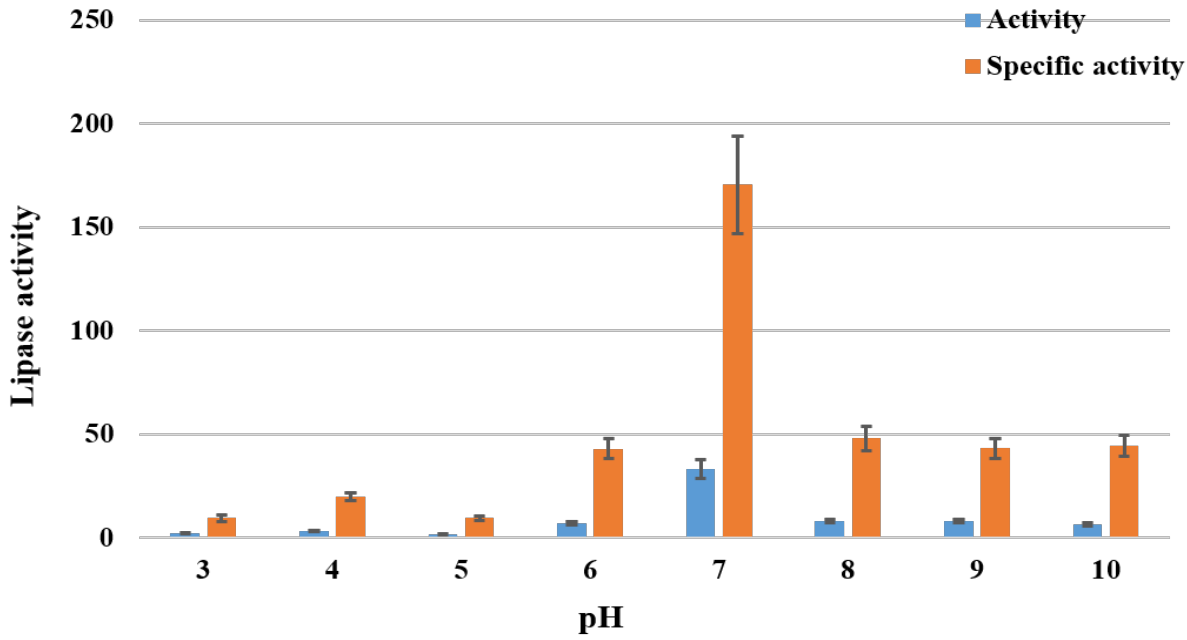

Figure51. Effect of medium's pH on the activity of cold-active lipase produced by *P. crustosum* AUMC 16061 at 20°C.

It had been found that the most active strain of *Fusarium solani* AUMC 16063 produced the highest levels of cold-active lipase at low pH (3.0) and temperature (15°C). To increase the synthesis of lipase, the nitrogen source and incubation period for this strain were optimized.

4.5. Optimization of nitrogen source and incubation time

F. solani AUMC 16063 was able to produce the maximum amount of lipase activity (46.66 U/mL/min) utilizing ammonium sulphate as a nitrogen source after 8 days of incubation at pH 3.0 and 15°C (Figure 52; Table 6). However, when yeast extract was employed as a nitrogen source, the generated cold-active lipase displayed the highest specific activity of 1550 U/mg protein (Figure 53; Table 6).

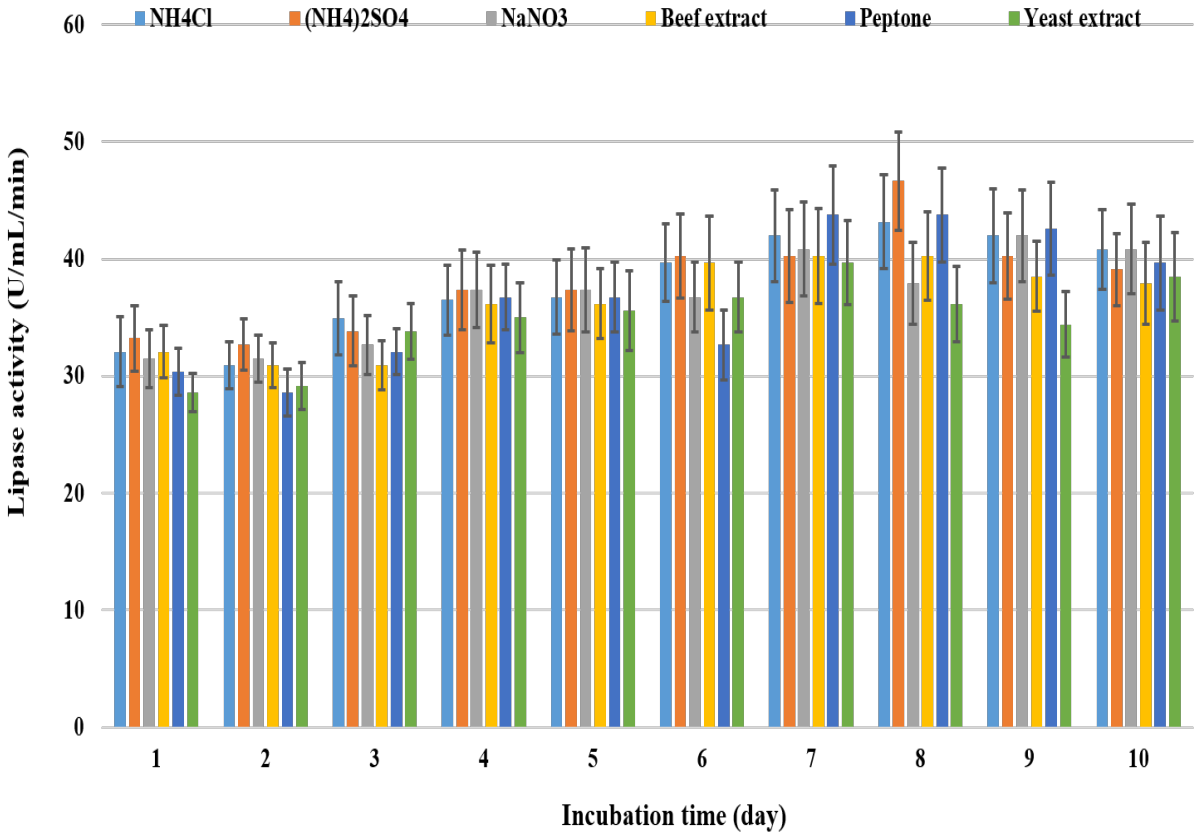

Figure 52. Effect of nitrogen source and incubation time on the activity of cold-active lipase produced by *F. solani* AUMC 16063 at 15°C.

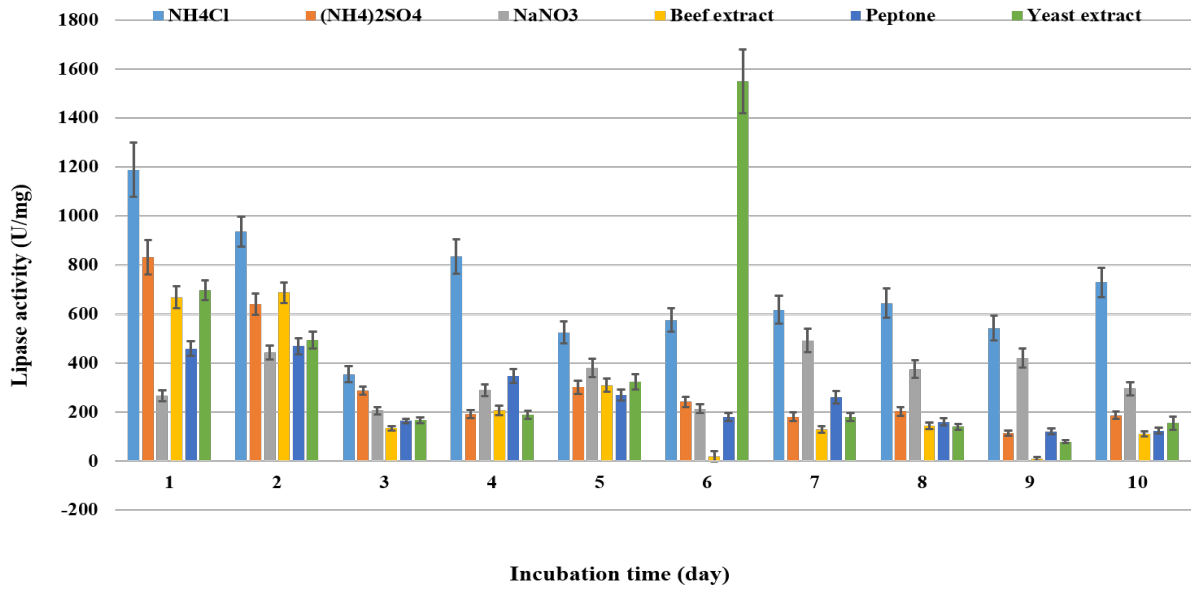

Figure 53. Effect of nitrogen source and incubation time on the specific activity of cold-active lipase produced by *F. solani* AUMC 16063 at pH 3.0 and 15°C.

Table 6. Optimization of Nitrogen source and incubation time of lipase production by *Fusarium solani* AUMC 16063

Nitrogen Sources	1 day		2 days		3 days		4 days		5 days	
	Lipase Activity (U/ml/min)	Specific Activity (U/mg)	Lipase Activity (U/ml/min)	Specific Activity (U/mg)	Lipase Activity (U/ml/min)	Specific Activity (U/mg)	Lipase Activity (U/ml/min)	Specific Activity (U/mg)	Lipase Activity (U/ml/min)	Specific Activity (U/mg)
NH_4Cl	32.08±3	1188.1±111	30.91±2	936.6±60.6	34.91±3.1	354.7±31.6	36.47±3	835±69.77	36.74±3.2	524.8±45.7
$(NH_4)_2SO_4$	33.24±2.8	831±70	32.66±2.2	640.3±43.1	33.83±3	286.6±16.6	37.33±3.4	191.4±17.4	37.33±3.5	301.0±28
$NaNO_3$	31.49±2.5	266.8±21.2	31.49±2	443.5±28.1	32.66±2.5	205.4±15.7	37.33±3.2	289.3±24.8	37.33±3.6	380.9±36.7
Beef extract	32.08±2.2	668.3±45.8	30.91±1.9	686.8±42.2	30.91±2.1	134.3±9.13	36.16±3.3	206.6±18.8	36.16±3	309.6±25.8
Peptone	30.33±2	459±30.3	28.58±2	468.5±32.8	32.08±2	163.6±10.2	36.74±2.8	346.6±28	36.74±3	270.1±22
Yeast extract	28.58±1.65	697.1±40.2	29.16±2	494.2±33.9	33.83±2.4	167.4±12	34.99±3	188.1±16.1	35.58±3.4	323.5±30.9

Nitrogen Sources	6 days		7 days		8 days		9 days		10 days	
	Lipase Activity (U/ml/min)	Specific Activity (U/mg)	Lipase Activity (U/ml/min)	Specific Activity (U/mg)	Lipase Activity (U/ml/min)	Specific Activity (U/mg)	Lipase Activity (U/ml/min)	Specific Activity (U/mg)	Lipase Activity (U/ml/min)	Specific Activity (U/mg)
NH_4Cl	39.66±3.3	574.7±47.8	41.99±3.9	617.5±57.3	43.16±4	644.1±59.7	41.99±4	542.8±51.9	40.83±3.4	729.1±60.7
$(NH_4)_2SO_4$	40.24±3.6	241.4±21.7	40.24±4	180.4±17.9	46.66±4.2	202.8±18.2	40.24±3.7	113.9±10.5	39.08±3.1	186.9±14.5
$NaNO_3$	36.74±3	213.6±17.4	40.83±4	491.9±48.2	37.91±3.5	375.3±35	41.99±3.9	419.9±39	40.83±3.8	295.8±27.5
Beef extract	39.66±4	198.3±20	40.24±4.1	129.8±13.2	40.24±3.8	143.2±13.5	38.49±3	100.1±7.8	37.91±3.5	112.4±10.4
Peptone	32.66±3	178.4±16.4	43.74±4.2	260.3±25	43.74±4	160.2±14.6	42.58±4	121.3±11.4	39.66±4	123.5±12.4
Yeast extract	36.74±3	1550.2±130	39.66±3.6	179.4±16.3	36.16±3.2	140.1±12.4	34.41±2.8	78.74±6.4	38.49±3.8	154.5±25.7

The cold-active lipase was produced by *F. solani* AUMC 16063 in solid state fermentation under the optimum conditions of a 8-day incubation period at pH 3.0 and 15℃ using $(NH_4)_2SO_4$as a nitrogen source. The enzyme was characterized in order to determine the optimum pH and temperature for the enzyme, the impact of various ions and inhibitors on the lipase activity, the best substrate and substrate concentration that gives the maximum lipase activity.

4.6. Determination of the optimum pH and temperature

The activity of the enzyme was evaluated at various pHs (3-11) and temperatures (5-20°C) in order to determine the optimum pH and temperature for the produced cold-active lipase. According to the current findings, lipase specific activity was at its highest (1052.6 U/mg) at pH 4.0 and 10°C (Figure 54; Table 7).

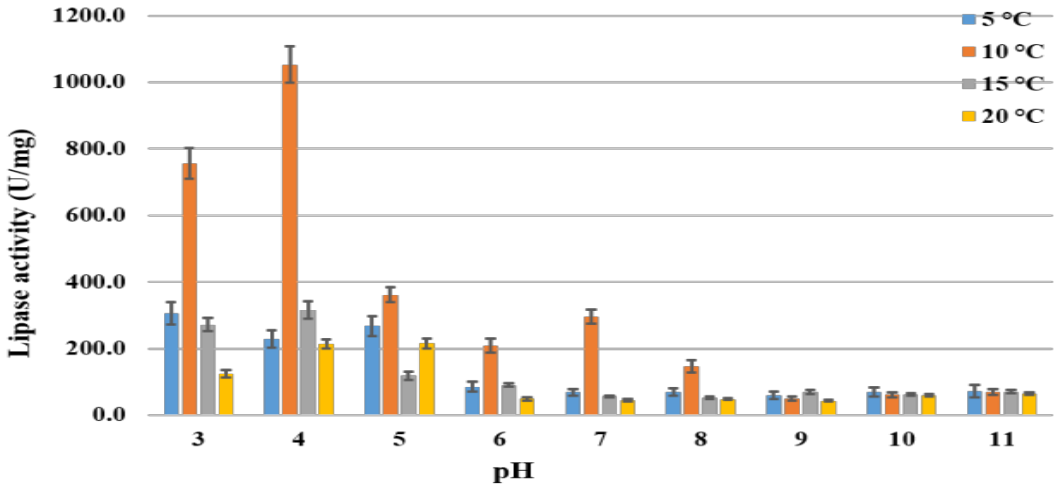

Figure 54. Effect of pH and temperature on the specific activity of the cold-active lipase produced by *F. solani* AUMC 16063.

Table 7. Effect of pH and temperature on the specific activity of the produced cold-active lipase

pH	5°C	10°C	15°C	20°C
3	305.9±34	756.6±46	271.4±20	123.36±11
4	228.6±26	**1052.6**±55	315.8±25	213.82±13
5	268.1±30	361.8±22	118.4±12	215.46±015
6	85.5±16	208.9±20	90.5±6	49.34±5
7	69.1±10	296.1±21	55.9±3	44.41±3
8	69.1±11	146.4±18	52.6±4	47.70±3
9	59.2±12	49.3±6	69.1±6	42.76±2.8
10	69.1±14	60.9±8	62.5±4	59.21±3.2
11	72.4±18	69.1±8	70.7±5	64.14±4

4.7. Effect of carbon source on lipase specific activity

In this investigation, the optimal substrate for the generated cold-active lipase was investigated using several oily substrates and tween. When tested at the optimum pH (4.0) and temperature (10°C) for the enzyme, tween 80 was shown to be the best, providing the peak (7400 U/g protein) of lipase activity (Table 8; Figure 55).

Table 8. Effect of different carbon source on the activity of the cold-active lipase produced by *F. solani* AUMC 16063 at pH 4.0 and 10°C.

Carbon source	Specific activity (U/mg protein)
Tween 20	5760.0±212
Tween 40	5430.0±216
Tween 80	**7400.0**±242
Olive oil	4280.0±200
Sunflower oil	1640.0±124
Maize oil	4280.0±226
Sesame oil	3780.0±140

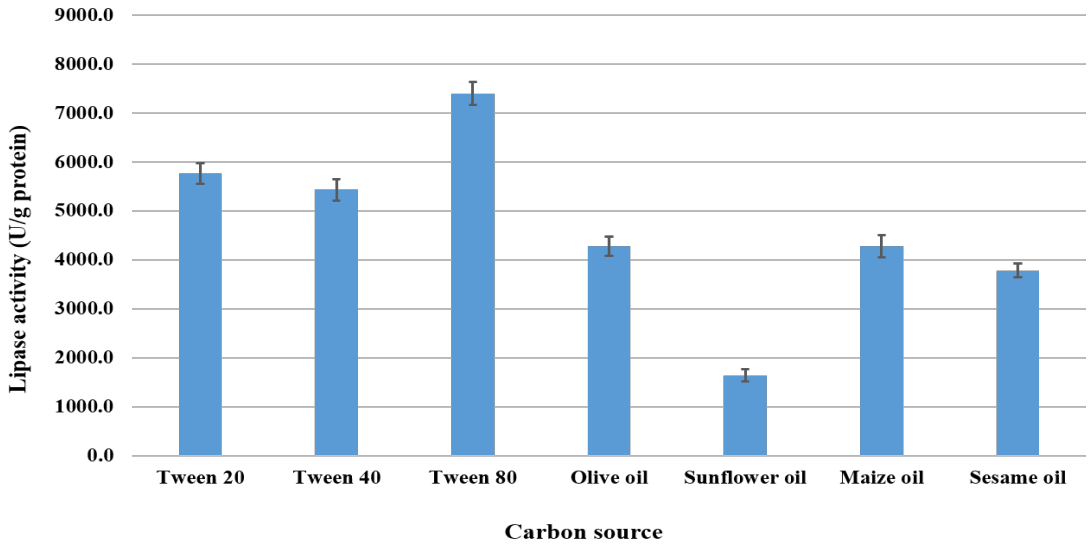

Figure 55. Effect of different oily substrates (carbon source) on the specific activity of the cold-active lipase (produced by *F. solani* AUMC 16063) at pH 4.0 and 10°C.

4.8. Effect of some ions and inhibitors on the lipase specific activity

The impact of certain ions was assessed using tween 80 as a substrate at pH 4 and 10°C. In this investigation, the cold-active lipase was activated by each of the examined ions. When

compared to the control, $CaCl_2$ had the highest activation impact (180%), while KCl had the lowest (102.3%) (Table 9; Figure 56).

Table 9. Effect of some ions and inhibitors on the activity of the cold-active lipase produced by *F. solani* AUMC 16063.

Ions and inhibitors	Specific activity (U/g protein)	Residual activity (%)
Control	**7400±242**	**100±3.27**
KCl	7570±300	102.3±4
$CuSO_4$	11180±528	151±7.1
NaCl	8880±500	120±6.75
$FeSO_4$	10030±560	135.54±7.56
$NiCl_2$	12010±680	162.3±9.2
$ZnSO_4$	10030±600	135.54±8.1
$MgSO_4$	9050±420	122.3±5.67
$CaCl_2$	13320±820	180±11.1
$CoCl_2$	8220±530	111±7.16
$MnSO_4$	9870±480	133.4±6.48
EDTA	9540±360	128.92±4.86
SDS	8220±300	111±4

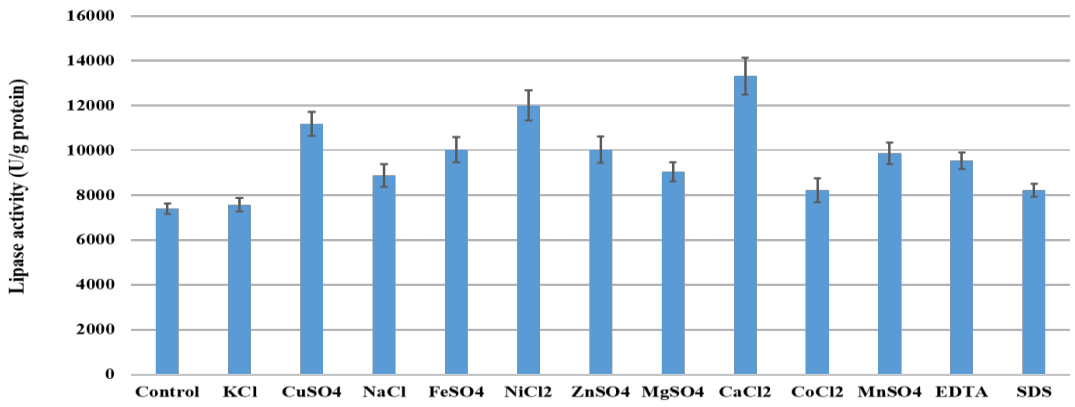

Figure 56. Effect of some monovalent and divalent ions, inhibitors and activators on the specific activity of the cold-active lipase produced by F. solani AUMC 16063 at pH 4.0 and 10°C.

4.9. Application of the purified cold-active lipase

The current study examined the potential of the cold-active lipase, in this study, for eliminating different oily spots. The observations revealed that, the oily spots were detached from the white cotton clothes after incubating the clothes with 50 U/mL of the cold-active lipase in the centre of the stain (corn, olive, sesame, and sunflower oil with Congo red dye) for 60 min (Figure 57). When the control (the oily stain without the addition of lipase) was compared to the treated cotton clothes, it became clear that the cold-active lipase, in this study, was more successful at removing oil dirt from the fibre surfaces.

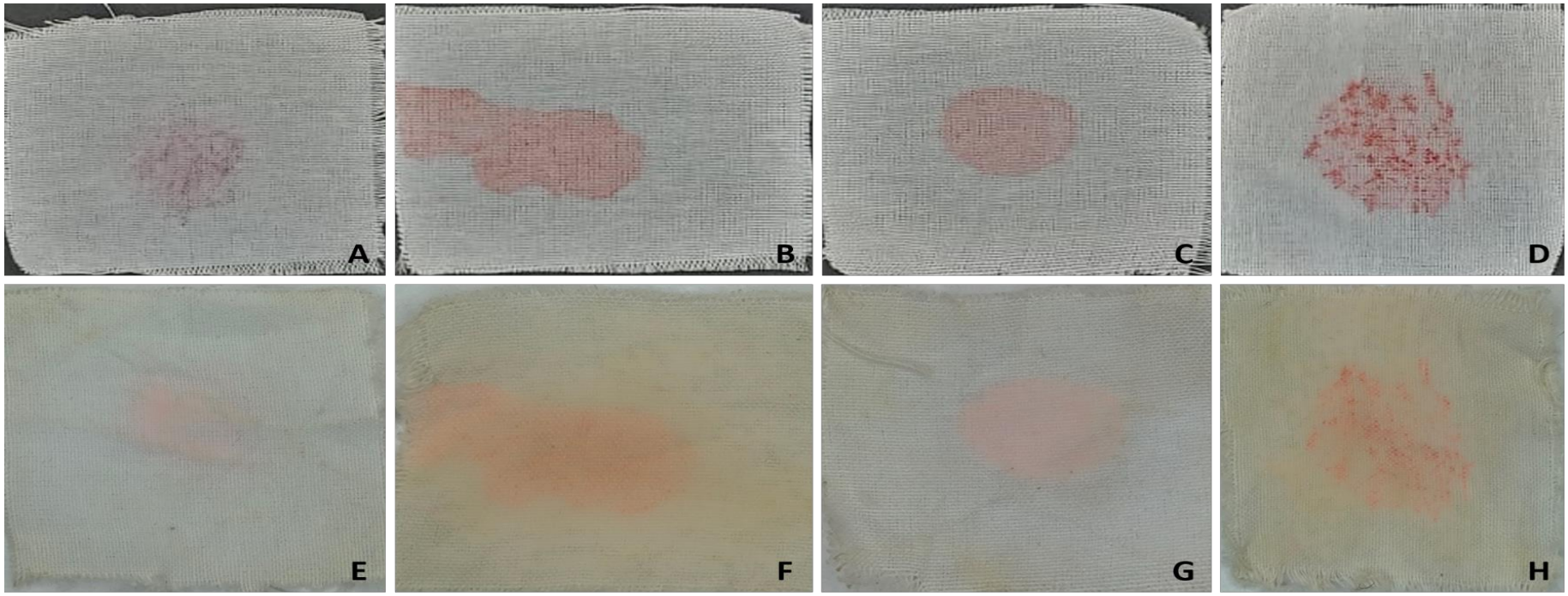

Figure 57. Removal of oily stains from cotton cloth pieces (5 cm × 5 cm) **(A–D),** Stained cotton cloth pieces with Corn, olive, sesame, and sunflower oil **(E–H),** Treated cotton cloth pieces (60 min, 150 rpm at pH 4.0 and 10°C) with the cold-active lipase (50 U/mL) produced by *Fusarium solani* AUMC 16063.

5.DISCUSSION

The goal of the current study was to identify the fungi that were present in seven composite samples of olive oil processing wastes (Fatura) that were collected in the Libyan cities of Zintan, Rayaina, Jadu, Yafran, Rujban, Al-Asabaa, and Gharyan. One of the most important duties to comprehend any bioremediation process is monitoring microbial communities. Only a few researches concentrating on the identification of microbial communities in olive mill wastes have been carried out, despite the fact that the necessity of monitoring microbial diversity has been widely discussed. Indeed, such research projects make it possible to analyze the biotransformations of olive mill waste in detail.To the best of our knowledge, this study is the first to focus on the isolation of fungi from Fatura and their usage in Libya to create cold-active lipases. The literature on biodiversity studies on the worldwide isolation of fungi from Fatura is very sparse.

In this study, 31 fungal species from 12 genera were isolated from these samples.*Fusarium* was the most common genus encountering 30.51% of total fungi. It was represented by two species, *F. oxysporum* and *F. solani*. *F. solani* was the most prevalent comprising 94% of total *Fusarium* and 28.7% of total fungi, while *F. oxysporum* encountered approximately 6.0% of total *Fusarium* and 1.8% of total fungi.

While fecal bacteria have also been detected, soil and freshwater habitats account for the majority of olive mill wastes (OMWs) microbiota (Kavroulakis and Ntougias 2011, Tsiamis *et al.* 2012). The particular cultivar from which OMWs are produced has a significant impact on the composition of bacterial communities (Tsiamis *et al.* 2012). Only 15% of the OTUs found in bacterial communities in OMW produced from distinct olive-fruit cultivars were shared, showing a cultivar-dependent microbial profile (Tsiamis *et al.* 2012).In all OMW samples examined by Tsiamis *et al.* (2012), the cultured bacterial diversity consisted of members of Firmicutes, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Gamma-proteobacteria and Bacteroidetes, while the implementation of a high density DNA microarray (PhyloChip) revealed a broader diversity, which was dominated by members of all classes of Proteobacteria, Firmicutes, Bacteroidetes, Chloroflexi, Cyanobacteria and Actinobacteria.

Yeast populationappears to be high in olive mill wastes (Sassi *et al.* 2006). Yeasts relatedto *Geotrichum*(*G. candidum*), *Candida*(*C. membranifaciens, C. michaelii, C. inconspicua,* and *C. tropicalis*), *Pichia*(*P. fermentans and P. holstii*), *Rhodotorula*(*R. mucilaginosa*),and Saccharomyces(*S. cerevisiae*)have been recently isolated from OMW (Bleve *et al.* 2011). In accordance, *Candida*

boidinii, Pichiaholstii(syn. *Nakazawaeaholstii*), *P. membranifaciens*, and*Saccharomyces cerevisiae*were the predominant yeasts in OMW from Apulia (Italy), exhibiting high pectolytic andxylanolytic activities. These yeast isolates could effectivelyreduce total phenolics, resulting in the reduction of severalphenolic compounds, in particular p-coumaric, vanillic and caffeic acids (Sinigaglia *et al.* 2010). *Pichia* (*P. guilliermondii*–syn. *Meyerozymaguilliermondii*) and *Candida* (*C. diddensiae*and*C. ernobii*)spp., were also the main yeast biota in OMW from Moroccanolive mills (Ben Sassi *et al.* 2008).

Pichia caribbica (syn. *Meyerozyma caribbica), P. holstii*(syn. *Nakazawaeaholstii),* and *Zygosaccharomycesfermentati (*syn. *Lachanceafermentati*) were the predominant yeast taxa in two-phase olive mill waste(TPOMW), while *Z. florentinus* (syn. *Zygotorulasporaflorentina*), *Lachanceathermotolerans* (syn. *Kluyveromycesthermotolerans*), *Saccharomyces cerevisiae,* and *S. rosinii* (syn. *Kazachstaniarosinii*) were minor constituents of the yeast community (Romo-Sánchez *et al.* 2010). Some of the yeast isolates from two-phase olive mill waste (TPOMW) exhibited cellulase, β-glucanase, β-glucosidase, peroxidase, andpolygalacturonase activities which could contribute to the degradation of complex compounds, including olive pomance phenolics. Based on the data provided by Romo-Sánchez *et al.* (2010), yeast diversity in olive pomance appears to be variety dependent.

A search of the National Center for Biotechnology Information (NCBI) turned up 106 deposited sequences of fungi found in waste environments at olive mills. The majority of the fungal species from olive mill wastes are classified as Glomeromycota, Basidiomycota, Ascomycota, and unclassified fungi, according to analysis of these sequences(Ntougias *et al.* 2013). More than 60% of the fungal species stored in GenBank are occupied by members of the Basidiomycota. Members of the Glomeromycota and unclassified fungi make up 19 and 17% of the records, respectively, while Ascomycota makes up just 3% of the total number of representatives. Since primers unique to Basidiomycota and Glomeromycota were used for the majority of the detected species, the Ascomycota population is actually underestimated (Ipsilantis *et al.* 2009, Karpouzas *et al.* 2009).

The potential to detoxify olive mill effluents has been found in members of the fungal genera *Acremonium*, *Alternaria*, *Aspergillus*, *Chalara*, *Fusarium*, *Lecythophora*, *Paecilomyces*, *Penicillium*, *Phoma*, *Phycomyces*, *Rhinocladiella*, and *Scopulariopsis* in OMW disposal ponds (Millán *et al.* 2000). However, rather than using molecular methods, these funguses were identified based on their morphology. Native microbiota that may break down OMW phenolics include those belonging to the fungal genera *Cerrena*, *Byssochlamys* (syn. *Paecilomyces*), *Lasiodiplodia*, and *Bionectria*, which were discovered using molecular methods (Mann *et al.* 2010).

Pichia, *Candida*, and *Saccharomyces*-like species are the most common yeasts in olive mill wastes, according to the studies cited above. Yeasts in olive mill wastes primarily act metabolically by reducing phenolics and sugars, albeit they appear to contribute less to OMW decolorization than white-rot fungi (Ben Sassi *et al.* 2008). Additionally, the waste from olive mills may have an advantage over bacteria due to their acidic pH. While white-rot fungi have been isolated to a lesser extent, filamentous fungus, such as *Aspergillus* and *Penicillium* spp., are frequent inhabitants of olive mill wastes (Millán *et al.* 2000, Aissam *et al.* 2007). It appears that the high levels of salt and sugar in olive mill wastes, along with the waste's acidic pH, encourage the establishment of osmotolerant yeasts in olive millwastes (Giannoutsou *et al.* 2004).

On lipase production agar medium at two temperatures, 10 and 20°C, 102 fungal isolates from 31 species related to 12 genera were tested for their lipolytic activity. The majority of fungi could produce lipase activity at 20°C, where 98 out of 102 isolates (96% of all isolates tested), were able to do so, compared to 73 isolates (71.56% of all isolates) at 10°C. While the number of intermediate lipase producers was wider at 20°C (71) than at 10°C (29), the number of the highest lipase producers was higher at 10°C (25) than at 20°C (16).*Aspergillus* species (8) were completely positive at 20°C compared to 4 positive species at 10°C, all *Penicillium* species (9) demonstrated positive findings at both of the temperatures examined, 10 and 20°C.

The understanding of cold-active lipases is expanding quickly, however research on cold active lipases is fragmented and lacking. No initiatives have been made to date to arrange this data. As a result, from the material found in the literature, an overview of this crucial enzyme for biotechnology and industry as well as its traits has been collected. It is evident from the scant reports on cold active lipases that have been published that the majority of research on these enzymes has been devoted to their isolation, purification, and characterization.

Physicochemical and nutritional parameters like pH, temperature, nitrogen source, and carbon source, have a significant impact on cold-active lipases production, which are primarily extracellular. The most active isolates in this study were *Alternaria alstroemeriae*, *Alternaria angustiovoidea*, *Fusarium solani*, and *Penicillium crustosum*. The four powerful fungal strains' production of cold-active lipase was maximized by optimizing some nutritional and environmental factors. *Fusarium solani* was found to be the best strain producing the most cold-active lipase. In literature, it was demonstrated that the majority of cold-active lipases are found in bacteria that can endure temperatures as low as 5°C. Even though there are several sources of lipase, only a few numbers of bacteria and

yeast were used to produce cold-active lipases. From time to time, efforts have been made to isolate these microbes' highly active lipases from cold environments.

In literature, no reports about production of cold-active lipase by *F. solani* were found. However, some other *Fusarium* species were used to produce cold-active lipases. Regarding this, Mase *et al.* (1995)purify and characterize lipase from *Fusarium* sp. YM-30. Knight *et al.* (2000)produced lipase enzyme from *F. solani* FS1. Quayson *et al.* (2020) produced lipase enzyme from *F. heterosporum*. Rifaat *et al.* (2010) produced lipase enzyme from *F. oxysporum*. Borges *et al.* (2021) developed lipase enzyme from *F. verticillioides* P24. The most popular technique for producing cold-active lipase is submerged fermentation (Dieckelmann *et al.* 1998; Lee *et al.* 2003). Microorganisms that are suited to the cold typically develop quickly when the temperature is low. According to Rashid *et al.* (2001), cold-active lipase synthesis is temperature-dependent and thermolabile.

In this study, *F. solani* AUMC 16063 utilized tween 80 and produce cold-active lipase at 15℃. Regarding this, tween 80 and Tributyrin induced production of cold active lipases at 4°C with an optimum pH 7.6 (Choo *et al.* 1998). *A. nidulans* WG312 produced cold active lipase by utilizing olive oil as an inducer at 30°C (Mayordomo *et al.* 2000). Soybean oil induced the production of cold active lipases from *Acinetobacter* sp. strain no. 6 at 4°C within four days (Suzuki *et al.* 2001). *Aeromonas* sp. LPB 4 produced lipase at 10°C in eight days' time duration by using tryptone and yeast extract as carbon and nitrogen source and trybutylin as an inducer (Lee *et al.* 2003). *Serratia marcescens* produced cold active lipase in presence of skim milk as energy source at 6°C in 6 days of incubation. Tween 80 and Tween 20 were the best inducers for cold-adapted lipase production with yeast extract as carbon source in 14 days at 25°C for *Psychrobacter* sp. wp37. Another isolate of *Pseudoalteromonas* sp. wp27 produced lipases at 25°C in 14 days with yeast extract as carbon source and olive oil and Tween 80 as inducers (Xiao *et al.* 2004).

The investigations on cold active lipases from *Psychrobacter immoblis* B10 were perused on semi-purified preparations, the nucleotide sequence of which is also available (Arpigny *et al.* 1993). Lipase purity is evaluated after each purification step by measuring the overall activity and specific activity. The purification efficiency is determined by total yield and purification factor (Joseph *et al.* 2008). For industrial applications the purification step should be economical, rapid, high yielding and easy to produce in large scale operations (Gupta *et al.* 2004). Prepurifaction step involves concentration of the protein containing lipases by ammonium sulphate precipitation, ultrafiltration or extraction with organic solvents.

Because these enzymes are inducible, their yield is determined by a variety of conditions. Culture practises (submerged or solid state fermentation), bioreactor design, and media composition are major determinants controlling lipase production. Carbon, nitrogen, and lipid sources primarily dictate the media requirements for optimal lipase synthesis for achieving high yield and maximum activity, which is critical in many industrial sectors. Several researches on optimizing lipase production by medium optimization have been designed with these considerations in mind.

The lipase production in the present study was found to be higher than many other fungal strains in literature. Regarding these findings, *T. pinophilus* AUN-1 produced the maximum lipase (4.11 U/mL) after 7 days' incubation at 30°C. On the 5th day of incubation at pH 6.0 and 20°C, *Penicillium chrysogenum* generated the highest lipase activity (68.0 U/mL) of culture media (Cho *et al.* 2007). On the 7th incubation day at pH 8.0 and 25°C, *Penicillium expansum* isolate SM3 lipase synthesis reached its maximum level (51.94 U/mL) (Mohammed *et al.* 2013).

Pandey *et al.* (2016)explored the lipase production by several *Penicillium* species, and their results were summarized as follows: on the 10th day at pH 5-7 and 15°C, *Penicillium cordubense* GBPP_P8 produced the most lipase (14.75 U/mL). The largest amount of *Penicillium raistrictum* GBPI_P98 lipase (23.50 U/mL) was produced on the 15th day of incubation at pH 5-7 and 15°C. *Penicillium raistrickii* GBPI_P101 produced the highest lipase (5.13U/mL) on the 15th day of incubation at pH 5-7 and 15°C. The largest amount of lipase (7.62 U/mL) was produced by *Penicilliumpolonicum* GBPI_P141 after 15 days of incubation at pH 5-7 and 15°C. On the 20th day of incubation, *Penicillium commune* GBPI_P150 produced the most lipase (4.02U/mL) at pH 5-7 and 25°C. The greatest lipase production (16.26U/mL) was displayed by *Penicilliumjensenii* GBPI_P188 on the 15th day of incubation at pH 6–9 and 15°C. The most lipase (22.82 U/mL) was produced by *Penicilliumaurantiovirens* GBPI_P222 on the 20th day of incubation at pH 5-7 and 15°C.

The pure lipase in this study was partially purified by ammonium sulphate precipitation, leading to a peak of specific activity of 1052.6U/mg at pH 4.0 and 10°C. The lipase used in this study is regarded as an extraordinarily extreme cold-active enzyme when compared to lipases produced by numerous fungi and bacteria. Regarding this, the lipase generated by *Penicillium canesense* BPF4 demonstrated the highest activity at pH 11 and 40°C (Sahay and Chouhan 2018), although its value was not provided. Purified lipase from *Bacillus methylotrophicus* PS3 displayed specific activity peak(693U/mg) at pH 7.0 and 50°C after a 2.90-fold purification (Sharma *et al.* 2017). *Bacillus pumilus* WSS5 was used to produce lipase that was most active at pH 8 and 37°C (Khan *et al.* 2023). Purified lipase from *Microbacterium* sp. showed its highest level of hydrolytic activity (4.9U/mg) at pH 8.5 and 50°C after 2.1-fold purification (Tripathi *et al.* 2014). From *Bacillus cereus* HSS, a cold-active

lipase with an ideal pH of 6.0 at 10°C was generated and purified, displaying a maximal activity of 632U/mg(Hassan *et al.* 2022). Cold-active lipase was created using *Pichia lynferdii* NRRL Y-7723 and showed its greatest activity (56,967.7 U/mg) at pH 8.0 at 15°C and pH 7.5 at 30°C (Bae *et al.* 2014).*Yarrowia lipolytica* LIPY8 produced a cold-active lipase with a peak activity (1102.9U/mg) at pH 7.5 and 17°C after 25.7-fold purification (Li *et al.* 2019).

It is believed that cold-active enzymes can slow down climate change by both lowering processing temperatures and producing the enzymes themselves using psychrophiles at lower temperatures (Sahay *et al.* 2013). Low activation energy requirements for the enzyme-substrate complex are a common characteristic of cold-adapted enzymes, which increase enzyme substrate affinity. This is achieved by modifying the flexibility of the enzyme structure, which increases the rate of enzymatic activity at low temperatures and hence lowers energy consumption. Currently, two theories are used to explain how cold-adapted enzymes catalyze: (1) The structure of the enzyme is more flexible structurally compared to mesophilic or thermophilic enzymes, (2) Because the enzyme requires less energy to activate than other molecules, it uses very little energy at low temperatures (Cavicchioli *et al.* 2011, Moharram *et al.* 2022).

In this investigation, the impact of various ions and inhibitors on the pure cold-active lipase's activity was assessed. The impact of certain ions was assessed using tween 80 as a substrate at pH 4 and 10°C. The cold-active lipase was activated by each of the examined ions. When compared to the control, $CaCl_2$ had the highest activation impact (180%), while KCl had the lowest (102.3%). Consistent with the present findings, the *Bacillus cereus* HSS lipase activity was most significantly impacted by $FeSO_4$ and KCl. Zn^{2+}, Cu^{2+}, and Ni^{2+} are examples of transition metals that significantly reduced enzyme activity, whereas EDTA increased. The addition of calcium increased the lipase activity of *Penicillium canesense* strains BPF4 and BPF6 by 300 and 280%, respectively. Cu^{2+} and Co^{2+} shown a marginal increase (31 and 38%) in *Pichia lynferdii* Y-7723's lipase activity.

Ions may affect protein stability through 'chemical' interactions (or chelation) with proteins to create complexes, such as when ions are utilized as an enzyme's co-substrate, substrate, or co-factor (Bauduin *et al.* 2004). The ability of an ion to alter the structure of water (referred to as a "physical" effect) was thought to be the main cause of its ion specificity (Bauduin *et al.*2004, Boström *et al.* 2004). Strongly hydrated ions that strengthen the structure of water are referred to as kosmotropes, whereas weakly hydrated ions that weaken the structure of water are referred to as chaotropes, or "structure-breakers." In contrast to chaotropes, which are often huge and low-charged, kosmotropes are typically small and highly charged. As a matter of fact, all multivalent ions are highly hydrated and kosmotropic(Krestov 1991, Zhao 2005).

The current study examined the potential of the cold-active lipase, in this study, for eliminating different oily spots. The observations revealed that, the oily spots were detached from the white cotton clothes after incubating the clothes with 50 U/mL of the cold-active lipase in the centre of the stain (corn, olive, sesame, and sunflower oil with Congo red dye) for 60 min. When the control (the oily stain without the addition of lipase) was compared to the treated cotton clothes, it became clear that the cold-active lipase, in this study, was more successful at removing oil dirt from the fibre surfaces.

Thesefindings indicate an obvious improvement in cleaning efficiency given the cold-active lipase in this study ability to completely remove toughstains. Currently, different types of lipases were used inmany modern types of heavy-duty powder and automaticdishwasher detergents in order to increase the detergencyand prevent scaling (Bora and Bora 2012). Besides, these enzymes have low toxicity as compared to the chemicalsused in conventional detergents and leave no harmfulresidues (Bharathi and Rajalakshmi 2019). In fact, severalstudies have reported the usefulness of thermostable alkaline lipases from *Bacillus sonorensis*(Nerurkar *et al.* 2013)and *Pseudomonas aeruginosa*(Grbavčić *et al.* 2011)for the removal of oily stains from the cotton fabric when mixed with detergent. In addition, the cold-active lipase from *B. subtilis*displayed good washing performance at low temperature (30°C) and alkaline pH(pH 8.0–11.0) (Eggert *et al.* 2002).

6.RECOMMENDATIONS

This investigation is the first to focus on the isolation of fungi from Fatura and their usage in Libya to create cold-active lipases. The literature on biodiversity studies on the worldwide isolation of fungi from Fatura is very sparse.and this is the first study in which the production, partial purification,maximized and characterization of a cold-active lipase enzyme by *Fusarium solani*.

Due to the importance of the study, we recommend conducting further studies on other fungal species that have achieved good results at producing lipase enzyme, paying attention to studying the biodiversity of fungal flora in the Libyan environment, and establishing a specialized research center in the field of fungal sciences, and a herbarium to preserved pure fungal isolates, especially isolates of economic and medical importance. We recommend encouraging and supporting researcher and institutions of scientific research.

7.SUMMARY

- In this study, seven composite samples of olive oil processing wastes (Fatura) were obtained from Zintan, Rayaina, Jadu, Yafran, Rujban, Al-Asabaa, and Gharyan. 31 fungal species from 12 genera were isolated from these samples with total CFUs of 29560.*Fusarium* was the most common genus encountering total CFUs of 9020 and comprising 30.51% of total fungi. It was represented by two species, *F. oxysporum* and *F. solani*. *F. solani* was the most prevalent comprising 94% of total *Fusarium* and 28.7% of total fungi, while *F. oxysporum* encountered approximately 6.0% of total *Fusarium* and 1.8% of total fungi.

- *Fusarium* was followed by *Aspergillus*, which made up 25.44% of all fungi and was represented by eight species. It appeared in all samples, which had a total CFU count of 7520. With 29.25% of all *Aspergillus* and 7.4% of all fungi, *Aspergillus aureolatus* was the most common species.

- After *Fusarium* and *Aspergillus*, *Penicillium* was ranked third. Nine different species were present. A total of 100% of samples were found to have CFUs of 5140 and 17.4% of all fungi. *Penicilliumaurantiogriseum* was the species that was found the most frequently across 6 of the 7 samples. 5.8% of all fungi and 33.85% of all *Penicillium* were present in it.

- The fourth most prevalent genus, which made up 14.14% of all fungi, was *Mucor*, which was represented by two known and one undetermined species. 4180 CFUs were discovered. The most common species, accounting for 88% of all *Mucor* and 11.9% of all fungi, was *M. hiemalis*.

- Eleven species related to seven genera comprising CFUs of 5700 were identified from Fatura collected from Zintan city at 25ºC. Species richness was pronounced in genus *Aspergillus*, where it included3 species (*A. aureolatus*, *A. niger* and *A. ustus*). It encountered 26.3% (1500 CFUs) of total counts of all fungi isolated. From these species, *A. niger* yielded the highest number of propagules (580 CFUs) followed by *A. ustus* (520) and *A. aureolatus* (400).

- *Penicillium* and *Alternaria* (2 species each) were the runner of *Aspergillus*. *Penicillium* (11.6 % of total fungi) was represented by *P. roquefortii* (10.5%) and *P. citrinum* (1%). *Alternaria*constituting 5.6% (320 CFUs) of total fungi. *A. alstroemeriae* and *A. angustiovoidea* (200 and 120 CFUs) respectively. On the other hand, the genus *Fusarium* was represented by one species only (*F. solani*) comprising the highest number of propagules (35.4% of total fungi). The remaining genera, *Microdochium*, *Mucor* and *Phialophora* were represented by one species each.

- Five genera represented by thirteen species were recovered from Rayaina sample. Species richness was pronounced in both *Aspergillus* and *Penicillium*, they were represented each by 4 species. The genus *Aspergillus* showed the highest CFUs (2020 colony out of 4260). The most common species was recovered in high CFUs was *A. fumigatus*. *Penicillium* follows *Aspergillus* in the number of colonies (780 out of 4260 colony). It was represented by *P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum* and *P. citrinum* (constituting 6.1 of total count of *Penicillium*). The results also showed that genus *Mucor* was represented by 3 species *(M. hiemalis*, *M. racemosus* and *Mucor* sp.) and harbored 12.2% of total count of fungi in this sample. Whereas *Fusarium* (*F. solani*) and *Stachybotrys* (*S. chartarum*) were recovered with one species each as shown.

- Four genera and 10 species were collected from Jadu in this investigation. Noticeably, genus *Penicillium*harbored the highest number of species (5) and total count of colony (2180 out of 5480, 39.8%). The genus represented by *P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum*, *P. citrinum* and *P. puberulum* with the most common was *P. aurantiogriseum*. *Aspergillus* (1120 CFUs, 20.4% of total count) was reported in two species *A. aureolatus* (5.8%) and *A. deflectus* (14.6%). In addition, *Fusarium* was represented by two species (*F. oxysporum* and *F. solani*), it was constituting high number of colonies (36.5% of total fungal count in Jadu). The fourth genus, *Phialophora* was isolated as *P. richardisae* (3.3% of total CFUs).

- In Yafran sample nine species related to seven genera were reported. Only genus *Penicillium* reported in 3 species (*P. aurantiogriseum*, *P. chrysogenum*and *P. expansum*) with 12.1% of total fungal colonies. The other remaining genera were represented by only one species. Regarding to the number of colonies forming units, *Mucor hiemalis*showed the highest (30.5%) followed by *Fusarium solani* (25.9%). The other species ranged from 2.8 to 14.2% of total count of fungi isolated from Yafran sample.

- Six genera presented by 11 species were recovered from Rujban sample, regarding the number of species, genus *Penicillium* was the first. It was reported in 4 species with *P. crustosum*is the most common (140 colony out of 400 *Penicillium* colonies). It is worth mentioning that, genus *Fusarium* (*F.oxysporum* and *F. solani*) was recovered in high CFUs (1960, 45% of total count). *Aspergillus* was the same in the number of species (*A. aureolatus* and *A. niger*), both species harbored 13.8% of total count. The other remaining genera (3) were presented in one species each (*Geotrichum candidum*, *Mucor hiemalis* and *Trichoderma harzianum*) and CFUs ranging from 5.5 to 15.6% of the fungal count percentage.

- Four genera and 8 species were encountered from Al-Asabaa sample. The most common genus was *Fusarium*, it was showed the highest count 40.7% of total fungal count (700 colonies out of 1720) and represented by 2 species (*F. oxysporum* and *F. solani*). *Aspergillus* follows *Fusarium* in number of colonies (represented by 2 species), it was encountered 29.1% of the total count. As shown in table (1), *Penicillium* was the most diverse genus, it was represented by 3 species. It was harbored 15.1% of the total fungal.

- Five genera including 11 species were totally collected from Gharyan sample. The most diverse genus was *Aspergillus*, recovered in 6 species, giving rise 55.83% of the total fungi. *A. fumigatus* the most common species, it was presented in 16.7% of *Aspergillus* count. *Penicillium* was reported in two species, *P. aurantiogriseum* and *P. griseofulvum*, giving rise 7.5% of the total fungi. The remaining species; *Fusarium solani*, *Mucor hiemalis* and *Myrothecium verrucaria* were emerged in 20.83, 8.34 and 7.5% of the total fungi.

- On lipase production agar medium at two temperatures, 10 and 20°C, 102 fungal isolates from 31 species related to 12 genera were tested for their lipolytic activity. The majority of fungi could produce lipase activity at 20°C, where 98 out of 102 isolates (96% of all isolates tested), were able to do so, compared to 73 isolates (71.56% of all isolates) at 10°C. While the number of intermediate lipase producers was wider at 20°C (71) than at 10°C (29), the number of the highest lipase producers was higher at 10°C (25) than at 20°C (16). *Aspergillus* species (8) were completely positive at 20°C compared to 4 positive species at 10°C, all *Penicillium* species (9) demonstrated positive findings at both of the temperatures examined, 10 and 20°C.

- The most active isolates were *Alternaria* (2 isolates), *Fusarium* (1), and *Penicillium* (1). Molecular identification of the most active four isolates was carried out by sequencing their internal transcribed spacer region (ITS). Pure cultures of the four fungal isolates were deposited in the culture collection of Assiut University Mycological Centre as *Alternaria* sp. AUMC 16060, *Alternaria* sp. AUMC 16062, *Fusarium* sp. AUMC 16063, and *Penicillium* sp. AUMC 16061.

- The four powerful fungal strains' production of cold-active lipase was maximized by optimizing some nutritional and environmental factors. For *Alternaria alstroemeriae*, *A. angustiovoidea*, and *Fusarium solani*, pH 3 produced cold-active lipase enzyme activity of 32.66, 31.5, and 33.83 U/mL/min, respectively, at 10°C. *Penicillium crustosum* performed best at pH 5, yielding 9.91 U/mL/min. The most potent strain was *Fusarium solani* when compared to the others. It generated the highest levels of specific activity (512.5 U/mg protein) and cold-active lipase activity (33.83 U/mL/min).

- At 15°C, pH 3 produced the best cold-active lipase enzyme activity for *Alternaria alstroemeriae*, *A. angustiovoidea*, and *Fusarium solani*, with values of 33.83, 39.66, and 45.49 U/mL/min, respectively. At pH 7, *Penicillium crustosum* produced 10.49 U/mL/min, which was its best output. Comparing the tested strains, *Fusarium solani* was the most powerful. It produced the highest levels of both cold-active lipase activity (45.49 U/mL/min) and specific activity (385.5 U/mg protein).

- At 20°C and pH 3.0, *Alternaria alstroemeriae* and *Fusarium solani* exhibited their highest levels of cold-active lipase activity (34.41 and 36.74 U/mL/min, respectively) and specific activity (296.63 and 854.4 U/mg protein, respectively). The cold-active lipase activity (22.16 U/mL/min) and specific activity (107.05 U/mg protein) of *Alternaria angustiovoidea* were at their highest levels at pH 4, respectively. The maximum cold-active lipase activity was, however, produced by *Penicillium crustosum* at pH 7.0, with 33.24 U/mL/min and 170.46 U/mL/min, respectively.

- It had been found that the most active strain of *Fusarium solani* AUMC 16063 produced the highest levels of cold-active lipase at low pH (3.0) and temperature (15°C). To increase the synthesis of lipase, the nitrogen source and incubation period for this strain were optimized.

- *F. solani* AUMC 16063 was able to produce the maximum amount of lipase activity (46.66 U/mL/min) utilizing ammonium sulphate as a nitrogen source after 8 days of incubation at pH 3.0 and 15°C. However, when yeast extract was employed as a nitrogen source, the generated cold-active lipase displayed the highest specific activity of 1550 U/mg protein after 6days of incubation.

- The cold-active lipase was produced by *F. solani* AUMC 16063 in solid state fermentation under the optimum conditions of a 8-day incubation period at pH 3.0 and 15°C using $(NH_4)_2SO4$as a nitrogen source. The enzyme was characterized in order to determine the optimum pH and temperature for the enzyme, the impact of various ions and inhibitors on the lipase activity, the best substrate and substrate concentration that gives the maximum lipase activity.

- The activity of the enzyme was evaluated at various pHs (3-11) and temperatures (5-20°C) in order to determine the optimum pH and temperature for the produced cold-active lipase. According to the current findings, lipase specific activity was at its highest (1052.6 U/mg) at pH 4.0 and 10°C.

- In this investigation, the optimal substrate for the generated cold-active lipase was investigated using several oily substrates and tween. When tested at the optimum pH (4.0) and temperature (10°C) for the enzyme, tween 80 was shown to be the best, providing the peak (7400 U/g protein) of lipase activity.

- The impact of certain ions was assessed using tween 80 as a substrate at pH 4 and 10°C. In this investigation, the cold-active lipase was activated by each of the examined ions. When compared to the control, $CaCl_2$ had the highest activation impact (180%), while KCl had the lowest (102.3%).

- This study looked at the possibility of using cold-active lipase to get rid of certain oily spots. After incubating the clothing with 50 U/mL of the cold-active lipase in the center of the stain (corn, olive, sesame, and sunflower oil with Congo red dye) for 60 minutes, the observations showed that the oily patches had separated from the white cotton clothing (Figure 55). It was evident from the comparison of the treated cotton clothing with the control (the oily stain without lipase addition) that the cold-active lipase in this investigation was more effective at eliminating oil dirt from the fiber surfaces.

8.REFERENCES

Abada, E.a.E.M., 2008. Production and characterization of a mesophilic lipase isolated from bacillus stearothermophilus ab-1. Pakistan Journal of Biological Sciences11 (8), 1100-1106.

Abd El-Sayed, E.S., El-Sakhawy, M., El-Sakhawy, M.a.-M., 2020. Non-wood fibers as raw material for pulp and paper industry. Nordic Pulp & Paper Research Journal35 (2), 215-230.

Aissam, H., Penninckx, M.J., Benlemlih, M., 2007. Reduction of phenolics content and cod in olive oil mill wastewaters by indigenous yeasts and fungi. World Journal of Microbiology and Biotechnology23, 1203-1208.

Akram, F., Mir, A.S., Haq, I.U., Roohi, A., 2023. An appraisal on prominent industrial and biotechnological applications of bacterial lipases. Molecular Biotechnology65 (4), 521-543.

Al-Bedak, O.A., Moharram, A.M., Hussein, N.G., Taha, D.M., Stephenson, S.L., Ameen, F., 2023. Microbial exploitation of feather wastes for sustainable production of keratinase and collagenase enzymes by didymella keratinophila aumc 15399 in submerged fermentation. Fermentation9 (6), 507.

Al-Bedak, O.A., Teama, E.A., Ali, E., Said, M., Shalaby, E., Moharram, Z.A., 2020. Impact of fumigation with phosphine on viability of wheat grains stored for six months at two levels of moisture content, in addition to description of four new records of associated fungi and assessment of their potential for enzymatic production. Journal of Basic & Applied Mycology (Egypt)11, 77-97.

Al-Doory, Y., 1980. Laboratory medical mycology. Lea and febiger. Philadelphia Kimpton Publishers, London.

Alabdalall, A.H., Alanazi, N.A., Aldakeel, S.A., Abdulazeez, S., Borgio, J.F., 2020. Molecular, physiological, and biochemical characterization of extracellular lipase production by *Aspergillus niger* using submerged fermentation. PeerJ8, e9425.

Ali, S., Khan, S.A., Hamayun, M., Lee, I. J., 2023. The recent advances in the utility of microbial lipases: A review. Microorganisms11 (2), 510.

Almeida, F.L., Travalia, B.M., Goncalves, I.S., Forte, M.B.S., 2021. Biodiesel production by lipase-catalyzed reactions: Bibliometric analysis and study of trends. Biofuels, Bioproducts and Biorefining15 (4), 1141-1159.

Ansorge-Schumacher, M.B., Thum, O., 2013. Immobilised lipases in the cosmetics industry. Chemical Society Reviews42 (15), 6475-6490.

Arpigny, J.L., Feller, G., Gerday, C., 1993. Cloning, sequence and structural features of a lipase from the antarctic facultative psychrophile *Psychrobacter immobilis* B10. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression1171 (3), 331-333.

Aruoma, O.I., 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis523, 9-20.

Bae, J.H., Kwon, M.H., Kim, I.H., Hou, C.T., Kim, H.R., 2014. Purification and characterization of a cold-active lipase from *Pichia lynferdii*Y-7723: pH-dependant activity deviation. Biotechnology and bioprocess engineering19, 851-857.

Bapiraju, K., Sujatha, P., Ellaiah, P., Ramana, T., 2005. Sequential parametric optimization of lipase production by a mutant strain *Rhizopus* sp. Btnt-2. Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms45 (4), 257-273.

Basheer, S.M., Chellappan, S., Beena, P., Sukumaran, R.K., Elyas, K., Chandrasekaran, M., 2011. Lipase from marine *Aspergillus awamori* BTMFW032:Production, partial purification and application in oil effluent treatment. New Biotechnology28 (6), 627-638.

Bauduin, P., Renoncourt, A., Touraud, D., Kunz, W., Ninham, B.W., 2004. Hofmeister effect on enzymatic catalysis and colloidal structures. Current opinion in colloid & interface science9 (1-2), 43-47.

Beisson, F., Arondel, V., Verger, R., 2000. Assaying arabidopsis lipase activity. Portland Press Ltd.

Ben Sassi, A., Ouazzani, N., Walker, G.M., Ibnsouda, S., El Mzibri, M., Boussaid, A., 2008. Detoxification of olive mill wastewaters by Moroccan yeast isolates. Biodegradation19, 337-346.

Benjamin, S., Pandey, A., 2001. Isolation and characterization of three distinct forms of lipases from *Candida rugosa* produced in solid state fermentation. Brazilian Archives of Biology and Technology44, 213-221.

Bharathi, D., Rajalakshmi, G., 2019. Microbial lipases: An overview of screening, production and purification. Biocatalysis and Agricultural Biotechnology22, 101368.

Bindiya, P., Ramana, T., 2013. Production of lipase by repeated batch fermentation using immobilized aspergillus sydowii. Int. J. Chem. Biochem. Sci4, 72-78.

Bleve, G., Lezzi, C., Chiriatti, M., D'ostuni, I., Tristezza, M., Di Venere, D., Sergio, L., Mita, G., Grieco, F., 2011. Selection of non-conventional yeasts and their use in immobilized form for the bioremediation of olive oil mill wastewaters. Bioresource Technology102 (2), 982-989.

Booth, C., 1971. The genus *Fusarium* commonwealth mycological institute. Kew, Surrey237.

Bora, L., Bora, M., 2012. Optimization of extracellular thermophilic highly alkaline lipase from thermophilic *Bacillus* sp. isolated from hotspring of Arunachal Pradesh, India. Brazilian Journal of Microbiology43, 30-42.

Borges, J.P., Quilles Junior, J.C., Ohe, T.H.K., Ferrarezi, A.L., Nunes, C.D.C.C., Boscolo, M., Gomes, E., Bocchini, D.A., Da Silva, R., 2021. Free and substrate-immobilised lipases from *Fusarium verticillioides* p24 as a biocatalyst for hydrolysis and transesterification reactions. Applied Biochemistry and Biotechnology193, 33-51.

Boström, M., Williams, D., Ninham, B., 2004. Why the properties of proteins in salt solutions follow a hofmeister series. Current opinion in colloid & interface science9 (1-2), 48-52.

Cai, Y., Wang, L., Liao, X., Ding, Y., Sun, J., 2009. Purification and partial characterization of two new cold-adapted lipases from mesophilic *Geotrichum* sp. SYBCWU-3. Process Biochemistry44 (7), 786-790.

Carvajal-Barriga, E.J., Portero Barahona, P., 2019. Production, extraction and characterization of lipases from the antarctic yeast *Guehomyces pullulans.* American Journal of Biochemistry and Biotechnology 15 (2): 75.82. DOI: 10.3844/ajbbsp.2019.75.82

Carvalho, R.S., Cruz, I.A., Américo-Pinheiro, J.H.P., Soriano, R.N., De Souza, R.L., Bilal, M., Iqbal, H.M., Bharagava, R.N., Ferreira, L.F.R., 2020. Interaction between *Saccharomyces cerevisiae* and *Lactobacillus fermentum* during co-culture fermentation. Biocatalysis and Agricultural Biotechnology29, 101756.

Cavicchioli, R., Charlton, T., Ertan, H., Omar, S.M., Siddiqui, K., Williams, T., 2011. Biotechnological uses of enzymes from psychrophiles. Microbial biotechnology4 (4), 449-460.

Chandra, P., Enespa, Singh, R., Arora, P.K., 2020. Microbial lipases and their industrial applications: A comprehensive review. Microbial cell factories19, 1-42.

- Cho, H.-Y., Bsncerz, R., Ginalska, G., Leonowicz, A., Cho, N.-S., Oga, S., 2007. Culture conditions of psychrotrophic fungus, *Penicillium chrysogenum* and its lipase characteristics. Journal of the Faculty of Agriculture Kyushu University 52(2):281-286.

Choo, D.-W., Kurihara, T., Suzuki, T., Soda, K., Esaki, N., 1998. A cold-adapted lipase of an alaskan psychrotroph, *Pseudomonas* sp. Strain b11-1: Gene cloning and enzyme purification and characterization. Applied and Environmental Microbiology64 (2), 486-491.

Choudhary, R., Jana, A., Jha, M., 2004. Enzyme technology applications in leather processing. Indin Journal of chemical technolog 11, 659-671.

Collins, T., Margesin, R., 2019. Psychrophilic lifestyles: Mechanisms of adaptation and biotechnological tools. Applied microbiology and biotechnology103, 2857-2871.

Colonia, B.S.O., Woiciechowski, A.L., Malanski, R., Letti, L.a.J., Soccol, C.R., 2019. Pulp improvement of oil palm empty fruit bunches associated to solid-state biopulping and biobleaching with xylanase and lignin peroxidase cocktail produced by *Aspergillus* sp. Lpb-5. Bioresource technology285, 121361.

Comménil, P., Belingheri, L., Sancholle, M., Dehorter, B., 1995. Purification and properties of an extracellular lipase from the fungus*Botrytis cinerea*.J Lipids30 (4), 351-356.

Cordova, J., Nemmaoui, M., Ismaıli-Alaoui, M., Morin, A., Roussos, S., Raimbault, M., Benjilali, B., 1998. Lipase production by solid state fermentation of olive cake and sugar cane bagasse. Journal of Molecular Catalysis B: Enzymatic5 (1-4), 75-78.

Criscuolo, A., Gribaldo, S., 2010. Bmge (block mapping and gathering with entropy): A new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC evolutionary biology10 (1), 210.

Cruz, Y.W., Vieira, Y.A., Vilar, D.S., Torres, N.H., Aguiar, M.M., Cavalcanti, E.B., Américo-Pinheiro, J.H., Soriano, R.N., Bharagava, R.N., Lima, Á.S., 2020. Pulp wash: A new source

for production of ligninolytic enzymes and biomass and its toxicological evaluation after biological treatment. Environmental Technology41 (14), 1837-1847.

Dalmau, E., Montesinos, J., Lotti, M., Casas, C., 2000. Effect of different carbon sources on lipase production by *Candida rugosa*. Enzyme and microbial technology26 (9-10), 657-663.

Das, A., Bhattacharya, S., Shivakumar, S., Shakya, S., Sogane, S.S., 2017. Coconut oil induced production of a surfactant-compatible lipase from *Aspergillus tamarii* under submerged fermentation. Journal of basic microbiology57 (2), 114-120.

Das, A., Shivakumar, S., Bhattacharya, S., Shakya, S., Swathi, S., 2016. Purification and characterization of a surfactant-compatible lipase from*Aspergillus tamarii*JGIF06 exhibiting energy-efficient removal of oil stains from polycotton fabric.3Biotech6, 1-8.

Dayanandan, A., Rani, S., Shanmugavel, M., Gnanamani, A., Rajakumar, G.S., 2013. Enhanced production of *Aspergillus tamarii* lipase for recovery of fat from tannery fleshings. Brazilian Journal of Microbiology44, 1089-1095.

De Azeredo, L.A., Gomes, P.M., Sant'anna, G.L., Castilho, L.R., Freire, D.M., 2007. Production and regulation of lipase activity from *Penicillium restrictum* in submerged and solid-state fermentations. Current Microbiology54, 361-365.

De María, P.D., Carboni-Oerlemans, C., Tuin, B., Bargeman, G., Van Gemert, R., 2005. Biotechnological applications of *Candida antarctica* lipase a: State-of-the-art. Journal of molecular catalysis b: enzymatic37 (1-6), 36-46.

De Souza, C.E.C., Ribeiro, B.D., Coelho, M.a.Z., 2019. Characterization and application of *Yarrowia lipolytica* lipase obtained by solid-state fermentation in the synthesis of different esters used in the food industry. Applied biochemistry and biotechnology189, 933-959.

Deak, T., Chen, J., Beuchat, L., 2000. Molecular characterization of *Yarrowia lipolytica* and *Candida zeylanoides* isolated from poultry. Applied and environmental microbiology66 (10), 4340-4344.

Diaz, J.M., Rodríguez, J., Roussos, S., Cordova, J., Abousalham, A., Carriere, F., Baratti, J., 2006. Lipase from the thermotolerant fungus *Rhizopus homothallicus* is more thermostable when produced using solid state fermentation than liquid fermentation procedures. Enzyme and Microbial Technology39 (5), 1042-1050.

Dieckelmann, M., Johnson, L., Beacham, I., 1998. The diversity of lipases from psychrotrophic strains of *Pseudomonas*: A novel lipase from a highly lipolytic strain of *Pseudomonas fluorescens*. Journal of Applied Microbiology85 (3), 527-536.

Dixit, S., Yadav, A., Dwivedi, P.D., Das, M., 2015. Toxic hazards of leather industry and technologies to combat threat: A review. Journal of Cleaner Production87, 39-49.

Dominguez De Maria, P., Carboni-Oerlemans, C., Tuin, B., Bargeman, G., Van Der Meer, A., Van Gemert, R., 2005. Biotechnological applications of *Candida antarctica* lipase a: State-of-the-art. Journal of molecular catalysis. B: Enzymatic37 (1-6), 36-46.

Domsch, K., Gams, W., Anderson, T., 2007. Compendium of soil fungi. 1–672. IHW-Verlag, Eching, Germany.

Dos Santos, M.M.O., Gama, R.S., De Carvalho Tavares, I.M., Santos, P.H., Gonçalves, M.S., De Carvalho, M.S., De Barros Vilas Boas, E.V., De Oliveira, J.R., Mendes, A.A., Franco, M.,

2021. Application of lipase immobilized on a hydrophobic support for the synthesis of aromatic esters. Biotechnology and Applied Biochemistry68 (3), 538-546.

Eggert, T., Van Pouderoyen, G., Pencreac'h, G., Douchet, I., Verger, R., Dijkstra, B.W., Jaeger, K.E., 2002. Biochemical properties and three-dimensional structures of two extracellular lipolytic enzymes from *Bacillus subtilis*. Colloids and Surfaces B: Biointerfaces26 (1-2), 37-46.

Elemuo, N.G., Ikiensikimama, S.S., Ubani, C.E., Egwim, E.C., Osuoha, J.O., 2019. Production and characterization of lipase enzyme expressed by crude oil contaminated soil isolates. Universal Journal of Microbiology Research7 (1), 1-6.

Ellis, M., 1976. More dematiaceous hyphomycetes (p. 507). Kew, Surrey, England: Commonwealth Mycological Institute.

Ellis, J., 1979. Preserving fungus strains in sterile water. Mycologia pp. 1072-1075.

Esakkiraj, P., Bharathi, C., Ayyanna, R., Jha, N., Panigrahi, A., Karthe, P., Arul, V., 2022. Functional and molecular characterization of a cold-active lipase from *Psychrobacter celer*PU3 with potential antibiofilm property. International Journal of Biological Macromolecules211, 741-753.

Espinel-Ingroff, A., Montero, D., and Martin-Mazuelos, E., 2004. Long-term preservation of fungal isolates in commercially prepared cryogenic microbank vials Journal of Clinical Microbiology (42), 1257–9

Essamri, M., Deyris, V., Comeau, L., 1998. Optimization of lipase production by rhizopus oryzae and study on the stability of lipase activity in organic solvents. Journal of Biotechnology60 (1-2), 97-103.

Fan, M., Zhang, P., Ma, Q., 2012. Enhancement of biodiesel synthesis from soybean oil by potassium fluoride modification of a calcium magnesium oxides catalyst. Bioresource Technology104, 447-450.

Farahat, S., Rabie, A., Farag, A., 1990. Evaluation of the proteolytic and lipolytic activity of different*Penicillium roqueforti* strains. Food chemistry36 (3), 169-180.

Fatima, S., Faryad, A., Ataa, A., Joyia, F.A., Parvaiz, A., 2021. Microbial lipase production: A deep insight into the recent advances of lipase production and purification techniques. Biotechnology and applied biochemistry68 (3), 445-458.

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution39 (4), 783-791.

Fernández-Bolaños, J., Rodríguez, G., Rodríguez, R., Guillén, R., Jiménez, A., 2006. Extraction of interesting organic compounds from olive oil waste. Grasasy aceites57 (1), 95-106.

Figueiredo, M., and C. Pimentel. 1975. Methods of preservation used in the Fungus culture collection of the Instituto Biologico [State of Sao Paulo; Brazil]. Summa Phytopathologica.

Geoffry, K., Achur, R.N., 2018. Screening and production of lipase from fungal organisms. Biocatalysis and agricultural biotechnology14, 241-253.

Gerday, C., 2014. Fundamentals of cold-active enzymes. Cold-adapted yeasts: biodiversity, adaptation strategies and biotechnological significance, 325-350.

Giannoutsou, E., Meintanis, C., Karagouni, A., 2004. Identification of yeast strains isolated from a two-phase decanter system olive oil waste and investigation of their ability for its fermentation. Bioresource Technology93 (3), 301-306.

Goddard, J.-P., Reymond, J.-L., 2004. Enzyme assays for high-throughput screening. Current opinion in biotechnology15 (4), 314-322.

Gopinath, S., Hilda, A., Ramesh, V., 1998. Detection of biodegradability of oils and related substances. Journal of environmental biology19 (2), 157-165.

Grbavčić, S., Bezbradica, D., Izrael-Živković, L., Avramović, N., Milosavić, N., Karadžić, I., Knežević-Jugović, Z., 2011. Production of lipase and protease from an indigenous *Pseudomonas aeruginosa* strain and their evaluation as detergent additives: Compatibility study with detergent ingredients and washing performance. Bioresource Technology102 (24), 11226-11233.

Grbavčić, S., Dimitrijević-Branković, S., Bezbradica, D., Šiler-Marinković, S., Knežević, Z., 2007. Effect of fermentation conditions on lipase production by *Candida utilis*. Journal of the Serbian Chemical Society72 (8-9), 757-765.

Gupta, N., Sahai, V., Gupta, R., 2007. Alkaline lipase from a novel strain burkholderia multivorans: Statistical medium optimization and production in a bioreactor. Process Biochemistry42 (4), 518-526.

Gupta, R., Gupta, N., Rathi, P., 2004. Bacterial lipases: An overview of production, purification and biochemical properties. Applied microbiology and biotechnology64, 763-781.

Hasan, F., Shah, A.A., Hameed, A., 2006. Industrial applications of microbial lipases. Enzyme and Microbial technology39 (2), 235-251.

Hassan, S.W., Abd El Latif, H.H., Beltagy, E.A., 2022. Purification, characterization and application of a cold active lipase from marine *Bacillus cereus* HSS.Microbiol. Biotechnol. Lett. 50(1), 71–80. http://dx.doi.org/10.48022/mbl.2109.09006

Hooda, S., 2020. Bio-processing and herbal treatment on textile: A route to sustainability. Int. J. Home Sci6, 285-288.

Houde, A., Kademi, A., Leblanc, D., 2004. Lipases and their industrial applications: An overview. Applied biochemistry and biotechnology118, 155-170.

Imamura, S., Takahashi, M., Misaki, H., Matsuura, K., 1989. Method and reagent containing lipases for enzymatic determination of triglycerides. West Germany Patent3 (912), 226.

Ipsilantis, I., Karpouzas, D.G., Papadopoulou, K.K., Ehaliotis, C., 2009. Effects of soil application of olive mill wastewaters on the structure and function of the community of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 41(12), 2466-2476.

Islam, R., Datta, B., 2015. Fungal diversity and its potential in environmental cleanup. Int J Res2, 815-825.

Izumi, T., Tamura, F., Akutsu, M., Katou, R., Murakami, S., 1997. Enzymatic transesterification of 3,7-dimethyl-4,7-octadien-1-ol, using lipases. Journal of Chemical Technology and

Biotechnology: International Research in Process, Environmental AND Clean Technology68 (1), 57-64.

Jadhav, V.V., Pote, S.S., Yadav, A., Shouche, Y.S., Bhadekar, R.K., 2013. Extracellular cold active lipase from the *Psychrotrophic halomonas* sp. Bri 8 isolated from the antarctic sea water. Songklanakarin Journal of Science & Technology35 (6).

Jaeger, K.-E., Reetz, M.T., 1998. Microbial lipases form versatile tools for biotechnology. Trends in biotechnology16 (9), 396-403.

Jamilu, H., Ibrahim, A.H., Abdullahi, S.Z., 2022. Isolation, optimization and characterization of lipase producing bacteria from abbatoir soil. Int. J. Adv. Sci. Technol3, 2708-7972.

Janssen, A.E., Sjursnes, B.J., Vakurov, A.V., Halling, P.J., 1999. Kinetics of lipase-catalyzed esterification in organic media: Correct model and solvent effects on parameters. Enzyme and microbial technology24 (8-9), 463-470.

Jayaprakash, A., Ebenezer, P., 2012. Purification and characterization of *Aspergillus japonicus* lipase from a pig fat production medium. Journal of Academia and Industrial Research1 (1), 1-7.

Ji, X., Li, S., Wang, B., Zhang, Q., Lin, L., Dong, Z., Wei, Y., 2015. Expression, purification and characterization of a functional, recombinant, cold-active lipase (lipa) from psychrotrophic *Yersinia enterocolitica*. Protein Expression and Purification115, 125-131.

Joseph, B., Ramteke, P.W., Thomas, G., 2008. Cold active microbial lipases: Some hot issues and recent developments. Biotechnology advances26 (5), 457-470.

Joseph, B., Upadhyaya, S., Ramteke, P., 2011. Production of cold-active bacterial lipases through semisolid state fermentation using oil cakes. Enzyme researchID 796407, 6 pages.doi:10.4061/2011/796407.

Karpouzas, D.G., Rousidou, C., Papadopoulou, K.K., Bekris, F., Zervakis, G.I., Singh, B.K., Ehaliotis, C., 2009. Effect of continuous olive mill wastewater applications, in the presence and absence of nitrogen fertilization, on the structure of rhizosphere–soil fungal communities. FEMS microbiology ecology70 (3), 388-401.

Katiyar, M., Ali, A., 2013. Effect of metal ions on the hydrolytic and transesterification activities of *Candida rugosa* lipase. Journal of Oleo Science62 (11), 919-924.

Katoh, K., Standley, D.M., 2013. Mafft multiple sequence alignment software version 7: Improvements in performance and usability. Molecular biology and evolution30 (4), 772-780.

Kavroulakis, N., Ntougias, S., 2011. Bacterial and β-proteobacterial diversity in *Olea europaea* var. Mastoidis-and *O. europaea* var. Koroneiki-generated olive mill wastewaters: Influence of cultivation and harvesting practice on bacterial community structure. World Journal of Microbiology and Biotechnology27, 57-66.

Kempka, A.P., Lipke, N.L., Da Luz Fontoura Pinheiro, T., Menoncin, S., Treichel, H., Freire, D.M., Luccio, M.D., De Oliveira, D., 2008. Response surface method to optimize the production and characterization of lipase from *Penicillium verrucosum* in solid-state fermentation. Bioprocess and Biosystems Engineering31, 119-125.

Khambhaty, Y., 2020. Applications of enzymes in leather processing. Environmental Chemistry Letters18 (3), 747-769.

Khan, F.I., Lan, D., Durrani, R., Huan, W., Zhao, Z., Wang, Y., 2017. The lid domain in lipases: Structural and functional determinant of enzymatic properties. Frontiers in bioengineering and biotechnology5, 16.

Khan, S.S., Verma, V., Rasool, S., 2023. Purification and characterization of lipase enzyme from endophytic *Bacillus pumilus*WSS5 for application in detergent industry. Biocatalysis and Agricultural Biotechnology50, 102681.

Khavitha, M., Shanthi, C., 2013. Isolation and characterization of cold active lipase producing *Pseudomonas* sp. 4 from marine samples of tamilnadu coast. Research Journal of Biotechnology l8, 4.

Kim, K.K., Song, H.K., Shin, D.H., Hwang, K.Y., Suh, S.W., 1997. The crystal structure of a triacylglycerol lipase from *Pseudomonas cepacia* reveals a highly open conformation in the absence of a bound inhibitor. Structure5 (2), 173-185.

Kirchman, D.L., Morán, X.a.G., Ducklow, H., 2009. Microbial growth in the polar oceans role of temperature and potential impact of climate change. Nature Reviews Microbiology7 (6), 451-459.

Knight, K., Pimentel, M.D.C.B., Morais, M.M.C.D., Ledingham, W.M., Lima Filho, J.L.D., Maia, M.D.M.D., 2000. Immobilization of lipase from *Fusarium solani*FS1. Brazilian journal of microbiology31, 219-221.

Ko, W., Wang, I., Ann, P., 2005. A simple method for detection of lipolytic microorganisms in soils. Soil Biology and Biochemistry37 (3), 597-599.

Krestov, G.A., 1991. Thermodynamics of solvation: Solution and dissolution, ions and solvents, structure and energetics. Ellis Horwood, New York, pp. 284.

Kumar, A., Gudiukaite, R., Gricajeva, A., Sadauskas, M., Malunavicius, V., Kamyab, H., Sharma, S., Sharma, T., Pant, D., 2020. Microbial lipolytic enzymes–promising energy-efficient biocatalysts in bioremediation. Energy192, 116674.

Kumar, A., Verma, V., Dubey, V.K., Srivastava, A., Garg, S.K., Singh, V.P., Arora, P.K., 2023. Industrial applications of fungal lipases: A review. Frontiers in Microbiology14, 1142536.

Kumar, D., Bhardwaj, R., Jassal, S., Goyal, T., Khullar, A., Gupta, N., 2021. Application of enzymes for an eco-friendly approach to textile processing. Environmental Science and Pollution Research 1-11.

Kumar, D., Kumar, L., Nagar, S., Raina, C., Parshad, R., Gupta, V.K., 2012. Screening, isolation and production of lipase esterase producing *Bacillus* sp. Strain DVl2 and its potential evaluation in esterification and resolution reactions. Archives of Applied Science Research4 (4), 1763-1770.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. Mega x: Molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution35 (6), 1547-1549.

Kurose, D., Seier, M.K., Evans, H.C., 2023. Exploiting exotic pathogens as mycoherbicides against invasive alien weeds: Japanese knotweed as a case study. Pest Management Science 80, 87–91

Lanka, S., Latha N. L. 2015. Purification and characterization of a new cold active lipase, EnL A from *Emericella nidulans* NFCCI 3643. African Journal of Biotechnology 14(22), 1897-1909. DOI: 10.5897/AJB2015.14674

Leal, M.C., Freire, D.M., Cammarota, M.C., Sant'anna Jr, G.L., 2006. Effect of enzymatic hydrolysis on anaerobic treatment of dairy wastewater. Process Biochemistry41 (5), 1173-1178.

Lee, H.K., Ahn, M.J., Kwak, S.H., Song, W.H., Jeong, B.C., 2003. Purification and characterization of cold active lipase from *Psychrotrophic aeromonas* sp. LPB 4. Journal of microbiology 41 (1), 22-27.

Leslie, J., Summerell, B., 2006. The *Fusarium* laboratory manual blackwell publishing. Ames, Iowa.

Li, C.Y., Cheng, C.Y., Chen, T.L., 2001. Production of *Acinetobacter radioresistens* lipase using tween 80 as the carbon source. Enzyme and microbial technology29 (4-5), 258-263.

Li, T., Zhang, W., Hao, J., Sun, M., Lin, S.-X., 2018. Cold-active extracellular lipase: Expression in SF9 insect cells, purification, and catalysis. Biotechnology Reports21, e00295.

Lima, L.R., Gonçalves, M.M., Couri, S., Melo, V.F., Sant'ana, G.F., Costa, A.D., 2019. Lipase production by*Aspergillus niger* by submerged fermentation. Brazilian Archives of Biology and Technology62.

Liu, X., Kokare, C., 2023. Microbial enzymes of use in industry. Biotechnology of microbial enzymes. Elsevier, pp. 405-444.

Lizardi-Jiménez, M., Hernández-Martínez, R., 2017. Solid state fermentation (Ssf): Diversity of applications to valorize waste and biomass. 3 Biotech7 (1), 44.

Lorenz, P., Liebeton, K., Niehaus, F., Eck, J., 2002. Screening for novel enzymes for biocatalytic processes: Accessing the metagenome as a resource of novel functional sequence space. Current opinion in biotechnology13 (6), 572-577.

Louwrier, A., 1998. Industrial products-the return to carbohydrate-based industries. Biotechnology and Applied Biochemistry27 (1), 1-8.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. Journal of biological chemistry193, 265-275.

Maharana, A. K., Ray, P. 2014. Screening of Psychrotrophic Micro-fungi for Cold Active Extracellular Enzymes Isolated from Jammu City, India. Journal of pure and applied microbiology 8 (3), 2369-2375.

Mahfoudhi, A., Benmabrouk, S., Fendri, A., Sayari, A., 2022. Fungal lipases as biocatalysts: A promising platform in several industrial biotechnology applications. Biotechnology and Bioengineering119 (12), 3370-3392.

Mahmoud, G.A., Koutb, M.M., Morsy, F.M., Bagy, M.M., 2015. Characterization of lipase enzyme produced by hydrocarbons utilizing fungus *Aspergillus terreus*. European Journal of Biological Research5 (3), 70-77.

Malilas, W., Kang, S. W., Kim, S. B., Yoo, H. Y., Chulalaksananukul, W., Kim, W. 2013. Lipase from *Penicillium camembertii* KCCM 11268: Optimization of solid state fermentation and application to biodiesel production. Korean J. Chem. Eng 30 (2), 405-412.
DOI:10.1007/s11814-012-0132-y

Mann, J., Markham, J., Peiris, P., Nair, N., Spooner-Hart, R., Holford, P., 2010. Screening and selection of fungi for bioremediation of olive mill wastewater. World Journal of Microbiology and Biotechnology26, 567-571.

Manurukchinakorn, S., Fujio, Y., 1997. Effect of enzymes on the degree of maceration of soybean fermented by *Rhizopus strains*. J. Fat. Agr., Kyushu Univ., 41 (3-4), 231-237.

Mase, T., Matsumiya, Y., Akiba, T., 1995. Purification and characterization of a new lipase from *Fusarium* sp. YM-30. Bioscience, biotechnology, and biochemistry59 (9), 1771-1772.

Mattedi, A., Sabbi, E., Farda, B., Djebaili, R., Mitra, D., Ercole, C., Cacchio, P., Del Gallo, M., Pellegrini, M., 2023. Solid-state fermentation: Applications and future perspectives for biostimulant and biopesticides production. Microorganisms11 (6), 1408.

Mauti, G.O., Onguso, J., Kowanga, D.K., Mauti, E.M., 2016. Biodegradation activity of *Aspergillus niger* lipase isolates from a tropical country garage.Journal of Scientific Research 5(1), 15-18.DOI:10.31254/jsir.2016.5104

Mayordomo, I., Randez-Gil, F., Prieto, J.A., 2000. Isolation, purification, and characterization of a cold-active lipase from *Aspergillus nidulans*. Journal of Agricultural and Food Chemistry 48 (1), 105-109.

Mehta, A., Bodh, U., Gupta, R., 2017a. Fungal lipases: A review. Journal of Biotech Research8, 58.

Mehta, A., Bodh, U., Gupta, R., 2017b. Fungal lipases: A review. Journal of Biotech Research8.

Mendes, D.B., Da Silva, F.F., Guarda, P.M., De Almeida, A.F., Guarda, E.A., 2022. Lipase from *Fusarium solani*: Optimization of culture conditions, biochemical properties, and production. Research, Society and Development 11 (11)e191111133447.

Millán, B., Lucas, R., Robles, A., García, T., De Cienfuegos, G.A., Gálvez, A., 2000. A study on the microbiota from olive-mill wastewater (OMW) disposal lagoons, with emphasis on filamentous fungi and their biodegradative potential. Microbiological research155 (3), 143-147.

Mohammed, S., Te'o, J., Nevalainen, H., 2013. A gene encoding a new cold-active lipase from an antarctic isolate of *Penicillium expansum*. Current genetics59, 129-137.

Moharram, A.M., Zohri, A.-N.A., Hesham, A.E.-L., Abdel-Raheam, H.E., Al-Ameen Maher, M., Al-Bedak, O.a.-H., 2022. Production of cold-active pectinases by three novel *Cladosporium* species isolated from egypt and application of the most active enzyme. Scientific Reports 12 (1), 1-17.

Mouad, A.M., Taupin, D., Lehr, L., Yvergnaux, F., Porto, A.L.M., 2016. Aminolysis of linoleic and salicylic acid derivatives with *Candida antarctica* lipase b: A solvent-free process to obtain amphiphilic amides for cosmetic application. Journal of Molecular Catalysis B: Enzymatic126, 64-68.

Moubasher, A., 1993. Soil fungi in qatar and other arab countries The Centre for Scientific and Applied Research, University of Qatar.

Moujehed, E., Zarai, Z., Khemir, H., Miled, N., Bchir, M.S., Gablin, C., Bessueille, F., Bonhommé, A., Leonard, D., Carrière, F., 2022. Cleaner degreasing of sheepskins by the *Yarrowia lipolytica*LIP2 lipase as a chemical-free alternative in the leather industry. Colloids and Surfaces B: Biointerfaces211, 112292.

Mukhtar, H., Khursheed, S., Mumtaz, M.W., Rashid, U., Al-Resayes, S.I., 2016. Optimization of lipase biosynthesis from *Rhizopus oryzae* for biodiesel production using multiple oils. Chemical Engineering & Technology 39 (9), 1707-1715.

Mukunda, S., Onkarappa, R., Prashith, K.T.,. 2012. Isolation and screening of industrially important fungi from the soils of Western ghats of Agumbe and Koppa, Karnataka, India. Science, Technology and Arts Research Journal (1), 27–32.

Nerurkar, M., Joshi, M., Pariti, S., Adivarekar, R., 2013. Application of lipase from marine bacteria *Bacillus sonorensis* as an additive in detergent formulation. Journal of surfactants and detergents 16 (3). 435-443.

Niaz, M., Iftikhar, T., Zia, M.A., 2013. Effect of nutritional factors on lipase biosynthesis by *Aspergillus niger* in solid state fermentation. Extensive Journal of Applied Sciences1 (1), 9-16.

Ntougias, S., Bourtzis, K., Tsiamis, G., 2013. The microbiology of olive mill wastes. Hindawi Publishing Corporation BioMed Research International Volume 2013, Article ID 784591, 16 pages.http://dx.doi.org/10.1155/2013/784591.

Ojo, A.O., De Smidt, O., 2023. Lactic acid: A comprehensive review of production to purification. Processes 11 (3), 688.

Oliveira, F., Moreira, C., Salgado, J.M., Abrunhosa, L., Venâncio, A., Belo, I., 2016. Olive pomace valorization by *Aspergillus* species: Lipase production using solid-state fermentation. Journal of the Science of Food and Agriculture96 (10), 3583-3589.

Pandey, N., Dhakar, K., Jain, R., Pandey, A., 2016. Temperature dependent lipase production from cold and pH tolerant species of *Penicillium*. Mycosphere 7 (10), 1533-1545.

Patel, G., Shah, K., 2020. Isolation, screening and identification of lipase producing fungi from cotton seed soapstock. Indian Journal of Science and Technology13 (36), 3762-3771.

Pérez, M.M., Gonçalves, E.C., Vici, A.C., Salgado, J.C., De Moraes Polizeli, M.D., 2019. Fungal lipases: Versatile tools for white biotechnology. Recent Advancement in White Biotechnology Through Fungi: Volume 1: Diversity and Enzymes Perspectives 361-404.

Pitt, J.I., 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and talaromyces. ,by John I. Pitt. Academic Press.

Pomeranz, Y., 2012. Functional properties of food components. Academic Press.

Posada, D., Crandall, K.A., 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics (Oxford, England)14 (9), 817-818.

Quayson, E., Amoah, J., Hama, S., Kondo, A., Ogino, C., 2020a. Immobilized lipases for biodiesel production: Current and future greening opportunities. Renewable and Sustainable Energy Reviews134, 110355.

Quayson, E., Amoah, J., Rachmadona, N., Morita, K., Darkwah, L., Hama, S., Yoshida, A., Kondo, A., Ogino, C., 2020b. Valorization of palm biomass waste into carbon matrices for the

immobilization of recombinant *Fusarium heterosporum* lipase towards palm biodiesel synthesis. Biomass and Bioenergy142, 105768.

Rahman, M., Hack-Polay, D., Billah, M.M., Un Nabi, M.N., 2020. Bio-based textile processing through the application of enzymes for environmental sustainability. International Journal of technology management & sustainable development 19 (1), 87-106.

Ramakrishnan, V., Goveas, L.C., Suralikerimath, N., Jampani, C., Halami, P.M., Narayan, B., 2016. Extraction and purification of lipase from *Enterococcus faecium* MTCC5695 by peg phosphate aqueous-two phase system (ATPS) and its biochemical characterization. Biocatalysis and agricultural biotechnology6, 19-27.

Rao, P.V., Jayaraman, K., Lakshmanan, C., 1993. Production of lipase by *Candida rugosa* in solid state fermentation. 2: Medium optimization and effect of aeration. Process Biochemistry 28 (6),391-395.

Raper, K.B., Fennell, D.I., 1965. The genus aspergillus. The genus *Aspergillus*. Williams and Wilkins, Baltimore, U.S. A.

Rashid, N., Shimada, Y., Ezaki, S., Atomi, H., Imanaka, T., 2001. Low-temperature lipase from psychrotrophic *Pseudomonas* sp. Strain KB700a. Applied and Environmental Microbiology 67 (9), 4064-4069.

Reetz, M.T., 2001. Combinatorial and evolution-based methods in the creation of enantioselective catalysts. Angewandte Chemie International Edition 40 (2), 284-310.

Rifaat, H.M., El-Mahalawy, A.A., El-Menofy, H.A., Donia, S.A., 2010. Production, optimization and partial purification of lipase from *Fusarium oxysporum*. Journal of Applied Sciences in Environmental Sanitation5 (1).

Rodríguez Couto, S., 2008. Exploitation of biological wastes for the production of value-added products under solid-state fermentation conditions. Biotechnology Journal: Healthcare Nutrition Technology 3 (7), 859-870.

Rodriguez, J., Mateos, J., Nungaray, J., González, V., Bhagnagar, T., Roussos, S., Cordova, J., Baratti, J., 2006. Improving lipase production by nutrient source modification using *Rhizopus* homothallicus cultured in solid state fermentation. Process Biochemistry 41 (11), 2264-2269.

Romo-Sánchez, S., Alves-Baffi, M., Arévalo-Villena, M., Úbeda-Iranzo, J., Briones-Pérez, A., 2010. Yeast biodiversity from oleic ecosystems: Study of their biotechnological properties. Food microbiology27 (4), 487-492.

Sadh, P.K., Duhan, S., Duhan, J.S., 2018. Agro-industrial wastes and their utilization using solid state fermentation: A review. Bioresources and Bioprocessing 5 (1), 1-15.

Sahay, S., Chouhan, D., 2018. Study on the potential of cold-active lipases from psychrotrophic fungi for detergent formulation. Journal of Genetic Engineering and Biotechnology16 (2), 319-325.

Sahay, S., Hamid, B., Singh, P., Ranjan, K., Chauhan, D., Rana, R., Chaurse, V., 2013. Evaluation of pectinolytic activities for oenological uses from psychrotrophic yeasts. Letters in applied microbiology57 (2), 115-121.

Salgado, C.A., Dos Santos, C.I.A., Vanetti, M.C.D., 2022. Microbial lipases: Propitious biocatalysts for the food industry. Food Bioscience45, 101509.

Salgado, J.M., Abrunhosa, L., Venâncio, A., Domínguez, J.M., Belo, I., 2016. Combined bioremediation and enzyme production by *Aspergillus* sp. In olive mill and winery wastewaters. International Biodeterioration & Biodegradation110, 16-23.

Salihu, A., Alam, M.Z., Alam, M.Z., 2012. Production and applications of microbial lipases: A review. Scientific Research and Essays7 (30), 2667-2677.

Santos, S., Puna, J., Gomes, J., 2020. A review on bio-based catalysts (immobilized enzymes) used for biodiesel production. Energies 13 (11), 3013.

Saranya, G., Ramachandra, T., 2020. Novel biocatalyst for optimal biodiesel production from diatoms. Renewable Energy153, 919-934.

Sassi, A.B., Boularbah, A., Jaouad, A., Walker, G., Boussaid, A., 2006. A comparison of olive oil mill wastewaters (OMW) from three different processes in Morocco. Process Biochemistry41 (1), 74-78.

Sethi, B.K., Rout, J.R., Das, R., Nanda, P.K., Sahoo, S.L., 2013. Lipase production by *Aspergillus terreus* using mustard seed oil cake as a carbon source. Annals of Microbiology63, 241-252.

Sharma, P., Sharma, N., Pathania, S., Handa, S., 2017. Purification and characterization of lipase by *Bacillus methylotrophicus*PS3 under submerged fermentation and its application in detergent industry. Journal of Genetic Engineering and Biotechnology15 (2), 369-377.

Sharma, S., Kanwar, S.S., 2014. Organic solvent tolerant lipases and applications. The Scientific World Journal, V 2014, Article ID 625258, 15 pages.http://dx.doi.org/10.1155/2014/625258

Silva, W.O.B., Mitidieri, S., Schrank, A., Vainstein, M.H., 2005. Production and extraction of an extracellular lipase from the entomopathogenic fungus *Metarhizium anisopliae*. Process Biochemistry 40 (1), 321-326.

Singh, A.K., Mukhopadhyay, M., 2012. Overview of fungal lipase: A review. Applied biochemistry and biotechnology166, 486-520.

Singhania, R.R., Patel, A.K., Soccol, C.R., Pandey, A., 2009. Recent advances in solid-state fermentation. Biochemical Engineering Journa l44 (1), 13-18.

Singhania, R.R., Sukumaran, R.K., Patel, A.K., Larroche, C., Pandey, A., 2010. Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme and Microbial Technology46 (7), 541-549.

Sinigaglia, M., Di Benedetto, N., Bevilacqua, A., Corbo, M.R., Capece, A., Romano, P., 2010. Yeasts isolated from olive mill wastewaters from southern Italy: Technological characterization and potential use for phenol removal. Applied microbiology and biotechnology87, 2345-2354.

Smith, N.R., Dawson, V.T., 1944. The bacteriostatic action of rose bengal in media used for plate counts of soil fungi. Soil science 58 (6), 467-472.

Smith D, and Onions A. H., 1994. The Preservation and Maintenance of Living Fungi 2nd ed (IMI Technical Handbooks) (USA: CABI).

Stahle, L., Wold, S., 1989. Analysis of variance (ANOVA). Chemometrics and intelligent laboratory systems 6 (4), 259-272.

Sumner, C., Krause, S., Sabot, A., Turner, K., Mcneil, C.J., 2001. Biosensor based on enzyme-catalysed degradation of thin polymer films. Biosensors and bioelectronics 16 (9-12), 709-714.

Supakdamrongkul, P., Bhumiratana, A., Wiwat, C., 2010. Characterization of an extracellular lipase from the biocontrol fungus, *Nomuraea rileyi*MJ, and its toxicity toward *Spodoptera litura*. Journal of Invertebrate Pathology 105 (3), 228-235.

Suzuki, T., Nakayama, T., Kurihara, T., Nishino, T., Esaki, N., 2001. Cold-active lipolytic activity of psychrotrophic *Acinetobacter* sp. Strain No; 6. Journal of bioscience and bioengineering 92 (2), 144-148.

Tan, J.S., Abbasiliasi, S., Ariff, A.B., Ng, H.S., Bakar, M.H.A., Chow, Y.H., 2018. Extractive purification of recombinant thermostable lipase from fermentation broth of *Escherichia coli* using an aqueous polyethylene glycol impregnated resin system. 3 Biotech8, 1-7.

Tan, T., Zhang, M., Wang, B., Ying, C., Deng, L., 2003. Screening of high lipase producing *Candida* sp. And production of lipase by fermentation. Process Biochemistry39 (4), 459-465.

Taskin, M., Ucar, M.H., Unver, Y., Kara, A.A., Ozdemir, M., Ortucu, S., 2016. Lipase production with free and immobilized cells of cold-adapted yeast *rhodotorula glutinis*HL25. Biocatalysis and Agricultural Biotechnology8, 97-103.

Teng, Y., Xu, Y., 2008. Culture condition improvement for whole-cell lipase production in submerged fermentation by rhizopus chinensis using statistical method. Bioresource technology99 (9), 3900-3907.

Tian, M., Fu, J., Wang, Z., Miao, C., Lv, P., He, D., Li, Z., Liu, T., Li, M., Luo, W., 2021. Enhanced activity and stability of rhizomucor miehei lipase by mutating n-linked glycosylation site and its application in biodiesel production. FUEl304, 121514.

Toida, J., Kondoh, K., Fukuzawa, M., Ohnishi, K., Sekoguchi, J., 1995. Purification and characterization of a lipase from *Aspergillus oryzae*. Bioscience, biotechnology, and biochemistry 59 (7), 1199-1203.

Toshihiko, K., Mari, T., Ishii, T., Iloth, Y., Kirimura, K., Usami, S., 1989. Production of lipase by *Rhizopus oligosporous* a newly isolated fungus. J. Hokoku Waseda 50 (125), 61-66.

Tripathi, R., Singh, J., Kumar Bharti, R., Thakur, I.S., 2014. Isolation, purification and characterization of lipase from *Microbacterium* sp. And its application in biodiesel production. Energy Procedia54, 518-529.

Tsiamis, G., Tzagkaraki, G., Chamalaki, A., Xypteras, N., Andersen, G., Vayenas, D., Bourtzis, K., 2012. Olive-mill wastewater bacterial communities display a cultivar specific profile. Current Microbiology64, 197-203.

Tuck, K.L., Hayball, P.J., 2002. Major phenolic compounds in olive oil: Metabolism and health effects. The Journal of nutritional biochemistry13 (11), 636-644.

Turati, D.F.M., Almeida, A.F., Terrone, C.C., Nascimento, J.M., Terrasan, C.R., Fernandez-Lorente, G., Pessela, B.C., Guisan, J.M., Carmona, E.C., 2019. Thermotolerant lipase from *Penicillium* sp. Section *Gracilenta cbmai* 1583: Effect of carbon sources on enzyme production, biochemical properties of crude and purified enzyme and substrate specificity. Biocatalysis and Agricultural Biotechnology17, 15-24.

Ullman, U., Blasins, G., 1974. A simple medium for the detection of different lipolytic activity of microorganisms. Zbl. Bakt. Hyg., II Abt. Orig. A229, 264-267.

Vakhlu, J., 2006. Yeast lipases: Enzyme purification, biochemical properties and gene cloning. Electronic Journal of Biotechnology9 (1), 0-0.

Van Reeuwijk, L., 2002. Procedures for soil analysis (international soil reference and information centre). Technical paper9, 120.

Vargas, G.D., Treichel, H., De Oliveira, D., Beneti, S.C., Freire, D.M., Di Luccio, M., 2008. Optimization of lipase production by *Penicillium simplicissimum* in soybean meal. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology83 (1), 47-54.

Vargas, V.A., Delgado, O.D., Hatti-Kaul, R., Mattiasson, B., 2004. Lipase-producing microorganisms from a Kenyan alkaline soda lake. Biotechnology letters26, 81-86.

Veerapagu, M., Narayanan, A.S., Ponmurugan, K., Jeya, K., 2013. Screening selection identification production and optimization of bacterial lipase from oil spilled soil. Asian J Pharm Clin Res6 (3), 62-67.

Verma, N., Thakur, S., Bhatt, A., 2012. Microbial lipases: Industrial applications and properties (a review). Int. Res. J. Biol. Sci1 (8), 88-92.

Vilar, D.S., Carvalho, G.O., Pupo, M.M., Aguiar, M.M., Torres, N.H., Américo, J.H., Cavalcanti, E.B., Eguiluz, K.I., Salazar-Banda, G.R., Leite, M.S., 2018. Vinasse degradation using pleurotus sajor-caju in a combined biological–electrochemical oxidation treatment. Separation and Purification Technology192, 287-296.

Wang, E.Q., Li, S.Z., Tao, L., Geng, X., Li, T.C., 2010. Modeling of rotating drum bioreactor for anaerobic solid-state fermentation. Applied Energy87 (9), 2839-2845.

Warcup, J., 1950. The soil-plate method for isolation of fungi from soil. Nature166 (4211), 117-118.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications18 (1), 315-322.

Winayanuwattikun, P., Kaewpiboon, C., Piriyakananon, K., Chulalaksananukul, W., Yongvanich, T., Svasti, J., 2011. Immobilized lipase from potential lipolytic microbes for catalyzing biodiesel production using palm oil as feedstock. African Journal of Biotechnology10 (9), 1666-1673.

Wongwatanapaiboon, J., Malilas, W., Ruangchainikom, C., Thummadetsak, G., Chulalaksananukul, S., Marty A., Chulalaksananukul W.,2016. Overexpression of *Fusarium solani* lipase in *Pichia pastoris* and its application in lipid degradation. Biotechnology & biotechnological equipment, 30 (5), 885-893. http://dx.doi.org/10.1080/ 13102818. 2016.1202779

Xiao, X., Wang, P., Wang, F., 2004. Screening and characterization of psychrotrophic, lipolytic bacteria fromdeep-sea sediments. Journal of microbiology and biotechnology14 (5), 952-958.

Yadav, K.S., Adsul, M., Bastawde, K., Jadhav, D., Thulasiram, H., Gokhale, D., 2011. Differential induction, purification and characterization of cold active lipase from *Yarrowia lipolytica*NCIM 3639. Bioresource technology102 (22), 10663-10670.

Yafetto, L., 2022. Application of solid-state fermentation by microbial biotechnology for bioprocessing of agro-industrial wastes from 1970 to 2020: A review and bibliometric analysis. Heliyon.

Yakubu, A., Saikia, U., Vyas, A., 2019. Microbial enzymes and their application in pulp and paper industry. Recent Advancement in White Biotechnology Through Fungi: Volume 3: Perspective for Sustainable Environments, 297-317.

Yang, F., Weber, T.W., Gainer, J.L., Carta, G., 1997. Synthesis of lovastatin with immobilized *Candida rugosa* lipase in organic solvents: Effects of reaction conditions on initial rates. Biotechnology and bioengineering56 (6), 671-680.

Yong, S. K., Lim, B. H., Saleh, L. 2016. Optimisation, purification and characterisation of extracellular lipase from *Botryococcus sudeticus* (UTEX 2629). Journal of Molecular Catalysis B: Enzymatic (126), 99–105.

Zeng, X., Xiao, X., Wang, P., Wang, R., 2004. Screening and characterization of psychrotrophic, lipolytic bacteria from deep-sea sediments. Journal of microbiology and biotechnology14 (5), 952-958.

Zhao, H., 2005. Effect of ions and other compatible solutes on enzyme activity, and its implication for biocatalysis using ionic liquids. Journal of Molecular Catalysis B: Enzymatic37 (1-6), 16-25.

المستخلص:

هدفت الدراسة الحالية إلى إنتاج وتنقية جزئية لليباز البارد النشط من بعض الفطريات المعزولة من مخلفات معالجة زيت الزيتون في الجبل الغربي بليبيا، 31 نوعًا فطريًا تنتمي إلى 12 جنسًا بإجمالي وحدات مكونة لمستعمرات فطرية قدرها 29560، وكان الفيوزاريوم هو الجنس الأكثر شيوعًا الذي سجل إجمالي 9020 وحدة تكاثر فطرية وشكل 30.51%من إجمالي الفطريات، وكانت فطرة *F. solani* هي الأكثر انتشاراً حيث شكلت 94%من إجمالي الفيوزاريوم و 28.7%من إجمالي الفطريات، تم اختبار 102 عزلة فطرية لفعاليتها في التحلل الدهني على وسط أجار إنتاج الليباز عند درجتي 10 و 20 درجة مئوية، وكانت العزلات الأكثر نشاطا هي *Alternaria* spp. (2)عزلة، *Fusarium* sp.، و *Penicillium* sp.، و تم إجراء التحديد الجزيئي للعزلات الأربعة الأكثر نشاطا من خلال تسلسلها (ITS)، عُظِمَ إنتاج السلالات الفطرية الأربعة القوية من الليباز البارد النشط من خلال تحسين بعض العوامل الغذائية والبيئية، وكانت السلالة *F. solani* AUMC 16063 قادرة على إنتاج أقصى قدر من نشاط الليباز (46.66 وحدة/مل/دقيقة) مع نشاط نوعي (202.8 وحدة/مجم) باستخدام كبريتات الأمونيوم كمصدر للنيتروجين بعد 8 أيام من الحضانة عند 15°م، ودرجة حموضة 3.0، وفي نفس الظروف بعد 6 أيام باستخدام مستخلص الخميرة كمصدر للنيتروجين، أظهر الليباز النشط البارد أعلى نشاط نوعي(1550 وحدة/مجم)، ونشاط ليباز (36.74 وحدة/مل/دقيقة)، هذه هي الدراسة الأولى التي يتم فيها إنتاج وتنقية جزئية، وتعظيم وتوصيف إنزيم الليباز النشط البارد بواسطة فيوزاريوم سولاني.

الكلمات الدالة: الإنزيمات النشطة الباردة، الليباز، فيوزاريوم سولاني، نشاط التحلل الدهني، النشاط النوعي.

دولة ليبيا
إدارة الدراسات العليا و التدريب
جامعة الزاوية – كلية العلوم
مركز الدراسات العليا و التدريب
قسم الأحياء - شعبة علم النبات

إنزيمات الليبيز النشطة عند درجات الحرارة المنخفضة و المنتجة بواسطة الفطريات المعزولة من المخلفات الصلبة لصناعة زيت الزيتون (الفيتورة) في الجبل الغربي - ليبيا

رسالة مقدمة إلى قسم النبات – كلية العلوم – جامعة الزاوية
لاستكمال متطلبات الحصول على درجة الماجستير في العلوم – نبات (علم الأحياء الدقيقة- فطريات)

مقدم من الطالبة
جهينة محمد الهادي جويلي
تحت إشراف الأستاذ الدكتور
محمد احمد ابوالقاسم الرياني
أستاذ الفطريات
قسم النبات – كلية العلوم
جامعة الزنتان
2023