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Characterization of Detergent-Compatible Lipases from *Candida albicans* and *Acremonium sclerotigenum* under Solid-State Fermentation

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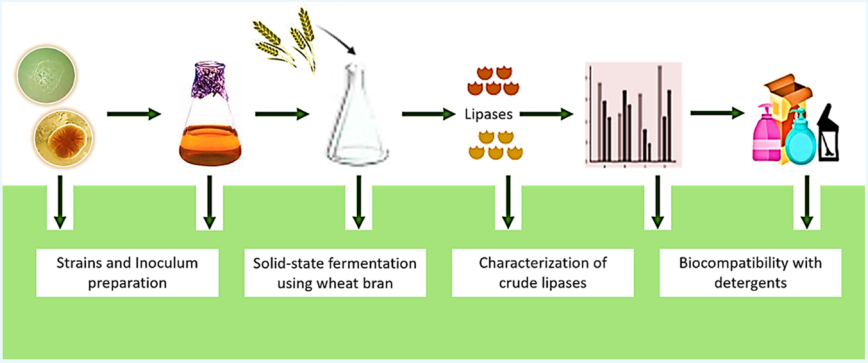

ABSTRACT: The purpose of this study was to compare and explore the potential of two distinct lipases at industrial levels after their production using wheat bran substrate in solid-state fermentation. Lipases from *Candida albicans* (*C. albicans*) and *Acremonium sclerotigenum* (*A. sclerotigenum*) were characterized to assess their compatibility and suitability for use in laundry detergents. The effects of pH, temperature, metal ions, inhibitors, organic solvents, and various commercially available detergents on these lipases were studied in order to compare their activity and stability profiles and check their stain removal ability. Both lipases remained stable across the wide pH (7−10) and temperature (30−50 °C) ranges. *C. albicans* lipase exhibited optimum activity (51.66 U/mL) at pH 7.0 and 37 °C, while *A. sclerotigenum* lipase showed optimum activity (52.12 U/mL) at pH 8.0 and 40 °C. The addition of Ca^{2+} and Mg^{2+} ions enhanced their activities, while sodium dodecyl sulfate (SDS) and ethylenediamine tetraacetic acid (EDTA) reduced their activities. Lipase from both strains showed tolerance to various organic solvents and considerable stability and compatibility with commercially available laundry detergents (>50%); however, *A. sclerotigenum* lipase performed slightly better. Characterization of these crude lipases showed nearly 60% relative activity after incubation for 2 h in various detergents, thus suggesting their potential to be employed in the formulation of laundry detergents with easy and efficient enzyme production. The production of thermostable and alkaline lipases from both strains makes them an attractive option for economic gain by lowering the amount of detergent to be used, thus reducing the chemical burden on the environment.

1. INTRODUCTION

Lipases, also known as triacylglycerol hydrolases (EC 3.1.1.3), are a group of enzymes that play a crucial role in the breakdown of triacyclglycerols, which are esters made up of glycerol and free fatty acids.[1] Due to their remarkable stability in organic solvents, pH, and temperatures, lipases are very effective at catalyzing interactions in aqueous as well as nonaqueous conditions.[2] Lipases are multifunctional enzymes that are extremely significant in industry because of their employment as a catalytic agent in numerous industrial applications, including butter and cosmetics production,[3] detergent supply,[4] polyester depolymerization, environmental cleanup,[5] the manufacture of saturated fatty acids after hydrolyzing fats and oils, triglyceride transesterification using shorter-chain alcohols to produce biodiesel,[6] the synthesis of fragrances, aromas, and flavors,[7] and the synthesis of vitamin C.[8] Their primary use is still as additives in washing detergents.[9]

Lipases have been discovered in several insect, animal, plant, and microbe species.[10] Lipases from fungal sources have received a great deal of attention because of their growing industrial applications. The major fungal genera involved in the synthesis of lipases include *Aspergillus*, *Rhizopus*, *Penicillium*,

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Candida, *Rhizomucor*, *Mucor*, *Geotrichum*, *Humicola*, and *Beauveria*.[11] These biocatalysts can be manufactured through liquid fermentation or solid-state fermentation (SSF). SSF is an effective method to reduce the cost of enzyme production, especially because it allows the use of agro-industrial residues as a medium. One advantage of solid-state fermentation for producing lipases is that the fermented solid material can be directly utilized as a biocatalyst after drying.[12] Agro-industrial waste products, called wheat brans, are good sources of proteins, minerals, lipids, and vitamins. These raw materials have been given additional value through the use of fermentation, particularly by SSF. These residues are not referred to as "wastes" because of their high nutritional content; rather, they are viewed as byproducts for the creation and development of other products.[13] The nutrients present in these byproducts provide suitable conditions for the development of microbes. The microbes have the capacity to ferment the source materials in order to reuse them. The agro-industrial byproducts are employed as a strong support for SSF advances to create a variety of useful products. SSF enables microorganisms to flourish in environments with low or minimal water content.[14]

The efficiency of enzymes is impacted by the stability of their structures. The stability of enzymes can be altered by changes in pH levels, as this affects the electrostatic interactions in the protein structure. The ionization state of the amino acids can be affected, leading to changes in secondary or tertiary structures in proteins. These structural changes can then impact the stability and activity of the enzymes.[15] Studies involving temperature and activity shed light on the stability of the active site. Enzyme activity may be lost if the active site is disturbed. Thermal inactivation at very high temperatures is a type of local unfolding (started as the temperature rises over the T_{opt}) leading to the denaturation or disruption of the tertiary structure of an enzyme.[16] Metal ions can interact with ionized fatty acids, affecting their behavior and solubility at interfaces. The rate at which fatty acids are released into the medium can be impacted by the presence of metal ions, which can affect the overall rate of the reaction.[17] Lipase inhibitors are substances that directly interact with the enzyme, preventing it from functioning properly and inhibiting its activity.[18]

Hydrolysis reactions that are catalyzed by lipase from various sources can be performed by using certain compounds to increase the area of the lipid−water interface. This, in turn, leads to an improvement in the rate of lipase-catalyzed reactions.[19] Organic solvents can have a direct impact on the shape of the active site of lipases, causing it to deform. Furthermore, alterations in pH levels and the formation of lipase protonated states can cause them to denature directly and lower their flexibility, thus affecting their structure and activity.[20] The continuous demand for lipase in industrial applications has made it the third most valuable group of enzymes in the market, following carbohydrases and proteases.[21] The free-form lipases are difficult to recover for reuse, are sensitive to changes in temperature and pH, and have low operating stability. Their usage is also technically constrained and more expensive.[22] The pursuit of new lipase sources is driven by many potential applications that require not just specificity of enzyme−substrate interactions but also stability of the process, such as thermal stability and a wide pH tolerance of the biocatalyst.[23] The purpose of this study was to assess the capacity of microbial lipases from two different sources, *Candida albicans* and *Acremonium sclerotigenum*, using solid-state fermentation. The current study utilized wheat bran (an agricultural byproduct) as a fermentation substrate. Solid-state fermentation was carried out with the microorganisms (*C. albicans* and *A. sclerotigenum*) grown on moistened solid substrates without excess water flow. SSF offered several advantages over regular submerged fermentation (SmF), such as cost-effectiveness, conservation of energy, decreased waste effluent issues, and enhanced product stability due to the reduced dilution of the medium. Optimal conditions, biochemical characterization, and stability profiles of the lipases from both strains were determined in order to promote their scale-up production. This study also explored the feasibility of incorporating enzymes in detergent formulations and the possibility of using them for destaining by lowering the quantity of detergent needed and the chemical burden of these detergents.

2. MATERIALS AND METHODS

2.1. Microorganisms and Chemicals. Strains of *C. albicans* and *A. sclerotigenum* were obtained from the Department of Biochemistry, The Islamia University of Bahawalpur, Pakistan.[24] Strains were cultured on Sabouraud Dextrose Agar (SDA) media plates consisting of (g/L): 40 g of dextrose, 10 g of peptone, and 20 g of agar, along with 0.5 g of chloramphenicol, and these plates with *C. albicans* and *A. sclerotigenum* cultures were incubated in an incubator (model FTC 90E, Usmate, Italy) for 5 days at 37 and 30 °C, respectively. Final storage was done at 4 °C and was periodically replicated every month. All of the apparatus, chemicals, and substrates used in experiments were of analytical grade and provided by the Department of Biochemistry, The Islamia University of Bahawalpur, Pakistan. Wheat bran (agro-industrial byproduct) was bought by local suppliers in Bahawalpur, Pakistan, and was dried, crushed, and served as the substrate of the growth medium.

2.2. Inoculum Preparation and Enzyme Production by SSF. Spores of *C. albicans* and *A. sclerotigenum* were collected aseptically from 5-day-old culture plates with a loop full of sporulation culture[25] and transferred into two separate 250 mL Erlenmeyer flasks containing Sabouraud Dextrose Broth (SDB: 40 g/L of dextrose, 10 g/L of peptone, final pH 7.0 at 25 °C). The flasks for *C. albicans* and *A. sclerotigenum* were then incubated for 72 h at 37 °C on a rotary shaker (model SI4, Shel Lab, Sheldon) operating at 120 rpm. Following this, SSF was performed in two separate 250 mL Erlenmeyer flasks for 7 days at 30 °C, using five grams of wheat bran moistened with 5% (v/w) mineral growth media in each flask. The mineral growth medium consisted of (g/L): 1 g of yeast extract, 2.0 g of KH_2PO_4, 12 g of NaH_2PO_4, 0.3 g of $MgSO_4{\cdot}7H_2O$, 10 g of $(NH_4)_2SO_4$, 0.25 g of $CaCl_2$, and 2% (v/v) olive oil per liter of solution. Both flasks were sterilized through autoclaving and inoculated separately with 5% (v/v) *C. albicans* and *A. sclerotigenum* inoculums, respectively. The flasks were then incubated for a week at 30 °C. The contents of the flasks were harvested by adding 100 mL of distilled water to each flask and shaking them for 30 min at 120 rpm. The mixture was filtered using muslin cloth and filter paper, respectively, and the resulting liquid was centrifuged using a high-speed centrifuge (model Z36HK, Hermle, Germany) at 12,000 rpm for 5 min. The crude lipases from both sources were then used for screening and activity determination.[26]

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ACS Omega 2023, 8, 32740−32751

2.3. Lipase Assays and Activity Measurements. The activity of crude lipases was assessed qualitatively by using a primary plate screening assay. The media composition for the preparation of agar plates consisted of 2% agar, 0.01% (w/v) methyl red indicator, and 2% (v/v) Tween-80. On each plate, a round well with a diameter of 1 cm was created. Crude lipase extract (10 μL) from *C. albicans* and *A. sclerotigenum* was poured into separate plates, and results were observed after 24 and 48 h. The fundamental idea underlying these treatments is the creation of distinct color variations that are caused by pH changes because of free fatty acids discharged from triacylglycerols throughout lipolysis.[27] Activities were measured quantitatively through a UV spectrophotometer (model LABINDIA UV-3000+) at 450 nm, with some modifications.[28] Crude lipase extract (200 μL) was added to 800 μL of substrate solution containing 80 mM $CaCl_2$ and 20 mM Tris-HCl (pH 8.5) with 3% (v/v) Tween-80. Tween-80 hydrolysis rate was directly observed by determining the variations in absorbance across a 5 min period.[28] Crude enzyme activities were determined in U/mL after all reactions were performed in triplicate. The maximal activities under optimal conditions without the addition of anything were considered to be under control. Relative enzyme activities were determined as the ratio of sample activity to control activity and thus presented as a percentage.[29] The following equation was used to define the relative activity of enzymes:

$$\text{Relative activity} = \frac{(A)t}{(A)o} \times 100\% \quad (1)$$

where $(A)t$ is the activity of the sample after the given time duration and $(A)o$ is the activity of the control under optimal conditions.

2.4. Characterization of *C. albicans* and *A. sclerotigenum* Crude Lipases. *2.4.1. Effect of Different pHs on Activity and Stability.* In order to investigate how pH affects enzyme activity, crude lipase extracts of *C. albicans* and *A. sclerotigenum* (200 μL each) were incubated for 2 h in buffers of several pH ranges, including citrate buffer (pH ranging from 5.0 to 6.0), tris-HCl buffer (pH ranging from 7.0 to 9.0), and glycine-NaOH buffer (pH ranging from 10.0 to 12.0), and their relative activities were measured. The optimal pH for each source was determined by plotting a graph between lipase activity and pH. The stability profile of enzymes was assessed, and results were presented in graphical form.[26]

2.4.2. Effect of Different Temperatures on Activity and Stability. The effect of different temperatures on *C. albicans* and *A. sclerotigenum* crude lipases was investigated by measuring their activity at various temperatures, including 20, 27, 30, 37, 40, 47, 50, and 55 °C, respectively. A graph of lipase activity vs temperature was used to determine the optimal temperatures. Thermal stabilities were also studied by preincubating the crude lipases for 2 h at temperatures of 20, 27, 30, 37, 40, 47, 50, and 55 °C, respectively, following the measurement of their relative activities.[30]

2.4.3. Effect of Activators and Inhibitors on Activity and Stability. The impact of various metal ions, including Mn^{2+}, Mg^{2+}, Ca^{2+}, K^+, and Na^+, and potent inhibitors such as sodium dodecyl sulfate (SDS) and ethylenediamine tetraacetic acid (EDTA), on lipase activity was determined by incubating the lipases in the presence of 1 mM of these metal ions or inhibitors. The stability of the lipases was evaluated by incubating them with 1 mM concentrations of metal ions and inhibitors for 2 h at 37 and 40 °C, respectively. After the incubation period, their relative activities were measured under the optimum assay conditions. A control sample without the addition of metal ions or inhibitors was also maintained under the same conditions.[31]

2.4.4. Effect of Organic Solvents on Activity and Stability. Organic solvents, including ethanol, methanol, acetone, diethyl ether, butanol, and isopropyl alcohol, were examined for their impacts on crude lipase activities by combining equal amounts of solvent and enzyme solution, and enzyme activities were immediately assessed under optimum assay conditions. To investigate the effect of solvents on the stability of crude lipases, equal amounts of solvents and crude lipases (1 mL each) were preincubated for 2 h at 37 and 40 °C, respectively, and their relative activities were measured. A control sample without the addition of solvents was also maintained under the same conditions.[32]

2.4.5. Effect of Laundry Detergents on Activity and Stability. The stability and activity of lipases from *C. albicans* and *A. sclerotigenum* in the presence of several commercial detergents that are sold in Pakistani markets, including Sufi, Brite, Ariel, Surf Excel, Express Power, and Bonus, were examined. In order to mimic washing conditions, detergents were dissolved in distilled water up to 7 mg/mL of the final volume. The enzymes that might have been included in detergent formulations were inactivated by heating them at 100 °C for 15 min. For the incubation of crude lipases from both strains, aliquots of these enzymes were incubated with an equal volume of each detergent solution (7 mg/mL) for 2 h at 37 and 40 °C, respectively. A control sample without the addition of laundry detergent was also maintained under the same conditions.[9]

2.4.6. Wash Performance Analysis in the Presence of Crude Lipase Extracts. The effectiveness of lipase as a biodetergent additive was investigated by testing its ability to remove various stains from 5 cm × 5 cm pieces of white cotton cloth. The cloth pieces were stained with grease, egg yolk, ketchup, olive oil, and butter stains. The objective was to determine the efficiency of lipase extracts in removing these stains and their impact on the overall cleaning efficacy. The results of this evaluation provided insights into the potential use of lipase as a sustainable additive to traditional detergents.[33] The stained cotton pieces were kept for 15 min at 120 rpm using 5 separate Erlenmeyer flasks (250 mL each) at 40 °C with various washing treatments as follows:

(a) Flasks with tap water (100 mL in each) and stained cloth pieces
(b) Flasks with tap water (100 mL in each), stained cloth pieces, and 1 mL of heat-inactivated detergent (7 mg/mL) in each flask
(c) Flasks with tap water (100 mL in each), stained cloth pieces, 1 mL of heat-inactivated detergent (7 mg/mL), and 1 mL of *C. albicans* lipase extract in each flask
(d) Flasks with tap water (100 mL in each), stained cloth pieces, 1 mL of heat-inactivated detergent (7 mg/mL), and 1 mL of *A. sclerotigenum* lipase extract in each flask

The treated cloth pieces were taken out after 15 min, washed with tap water, dried, and then subjected to a visual inspection in order to determine how well the enzymes removed the stains.

2.5. Statistical Analysis. All tests were carried out in triplicate, with controls under the same standardized

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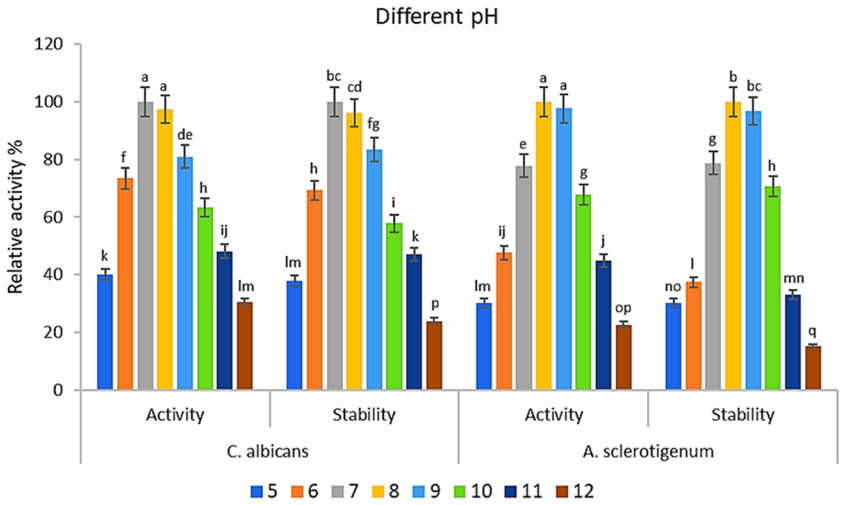

Figure 1. Effect of different pHs on the activity and stability of lipases from *C. albicans* and *A. sclerotigenum*. The pH profiles were determined using different buffer solutions with varying pH from 5.0 to 12. The maximum activities obtained at pH 7.0 and 8.0 for *C. albicans* and *A. sclerotigenum* lipases, respectively, were considered 100%. The pH stabilities of lipases were determined by incubating enzymes of both strains in a pH range of 5.0−12 for 2 h at 37 and 40 °C, respectively, and relative enzyme activities were measured using Tween-80 substrate. Each point in the graph shows the mean obtained from three individual experiments. The vertical bars represent the mean's standard error. Means having different letters are statistically different ($p < 0.05$), while means sharing the same letters are statistically at par with each other.

experimental conditions as the experiments. The findings of the data were represented as the mean and ± standard deviation (SD). To determine the group of means by analysis of variance (ANOVA), Tukey's honestly significant difference (HSD) test was employed following the comparison of means. Statistical significance was defined as ($p < 0.05$). Statistics 8.1 software was used for statistical analysis, and Microsoft Excel was used to make graphs.

3. RESULTS AND DISCUSSION

3.1. Lipase Production and Assays. Solid-state fermentation (SSF) was carried out for the production of lipase extracts from both strains using a wheat bran substrate. To confirm the presence of lipase from *C. albicans* and *A. sclerotinum*, a primary plate screening assay was performed using Tween-80 substrate and methyl red indicator. Observations showed that after 24 h, a color change from red to pink or purple occurred in the surroundings of the well carrying the crude enzyme extract, and these regions enlarged after 48 h. This color change was due to the change in pH that took place as a result of lipolysis, which caused the production of free fatty acids from triacylglycerols. Thus, this method is a quick and accurate way to check the lipolytic activity of crude lipase extracts. Lipase activities, when measured spectrophotometrically, *C. albicans* lipase showed 51.66 U/mL of enzyme activity at optimum conditions (pH 7.0, 37 °C), while *A. sclerotigenum* lipase showed 52.12 U/mL of enzyme activity at optimum conditions (pH 8.0, 40 °C). These maximal activities, obtained under optimal conditions, were considered 100%.

SSF is a cost-effective and efficient method for the production of fungal, yeast, and mycelial lipases on large scales. This process is an alternative to submerged fermentation and has become a preferred method due to its lower cost and simplicity. In SSF, microorganisms are grown on the surface of a solid substrate, where nutrients are absorbed, and moisture does not exceed the water retention capacity of the matrix. This approach provides a stable environment for the growth and production of lipases by fungi.[34] The SSF utilizing *Aspergillus niger* yielded crude lipase with an activity of 10.83 U/mL.[35] The production of fungal lipase is significantly influenced by the nitrogen source. Wheat bran comprises 13.1 ± 1.3% protein on average, and several authors have reported it as a protein-rich solid substrate for the production of fungal lipase.[36] Olive oil is one of the most frequently used substrates in the production of lipases by filamentous fungi. This substrate served a dual purpose, as it not only provided a source of carbon for the growth of microbes but also acted as a potent inducer for lipase production.[37] Utami et al. demonstrated that when 4, 8, and 2% olive oil were added to solid-state fermentation, the activity of lipase from *A. niger* was greater in the 2% concentration of olive oil.[38] Lipolytic microbes can be screened by using solid agar media with additional substrates and indicator dyes. It is a beneficial and quick screening method for the identification of specific lipase-producing microbes.[39] In lipase screening assays, surfactants known as tweens are often preferred as substrates due to their ease of integration in culture medium and their ability to facilitate optimal interaction between the cells or enzyme and the substrate.[40] A mutant strain from *Candida antarctica* DSM-3855 produced the highest lipase yield of 27.34 U/mL after incubation for 58 h under optimal conditions (pH 6.0, 26 °C).[41] *C. albicans* exhibited maximal production of lipase (410 U/mL) at pH 5.2, 30 °C; *A. flavus* (460 U/mL) at pH 6.2, 37 °C; and *Pseudomonas aeruginosa* (400 U/mL) at pH 6.2, 30 °C, after incubation for 5 days.[42] *Candida albicans* was found to produce both extracellular and intracellular lipases in the media tested. The highest enzyme activity was observed after 24 h of growth, although lipase production was detected throughout the entire growth phase. The optimal temperature and pH for lipase activity were determined to be 37 °C and 7.0, respectively.[43] *Acremonium*-like ROG 2.1.9 lipases restored around 85% of enzyme activity after an incubation of 6 h at 55 °C.[44] A gene designated *"lipA"* was identified in the *Acremonium alcalophilum* genome, which encodes a protein with a putative lipase domain. This gene was found to produce a novel lipolytic enzyme, LipA, which exhibited both acetylxylan esterase and lipase activity. LipA was

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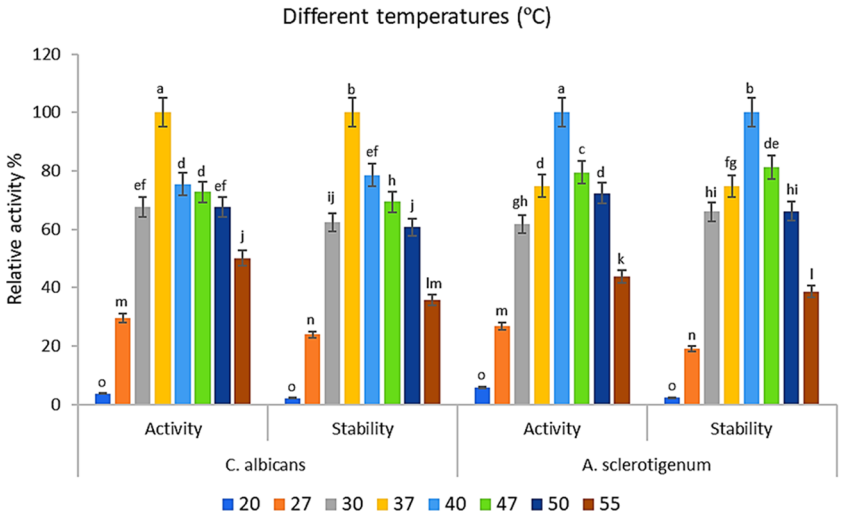

Figure 2. Effect of different temperatures on the activity and stability of lipases from *C. albicans* and *A. sclerotigenum*. Temperature profiles were determined by analyzing enzymatic activities at different temperatures and pH 7.0 for *C. albicans* lipase and pH 8.0 for *A. sclerotigenum* lipase. The enzymatic activities at 37 °C (for *C. albicans* lipase) and at 40 °C (for *A. sclerotigenum* lipase) were taken as 100%. The temperature stabilities were determined by incubating lipases from both strains at varying temperatures. The relative enzyme activities were measured at optimal conditions using Tween-80 substrate. Each point in the graph shows the mean obtained from three individual experiments. The vertical bars represent the standard error of the mean. Means having different letters are statistically different ($p < 0.05$), while means sharing the same letters are statistically at par with each other.

characterized as a glycoprotein with optimal pH and temperature ranges of 8.0 and 40 °C, respectively.[45] The study found that *C. albicans* and *A. sclerotigenum* possess the ability to synthesize extracellular crude lipases.

3.2. Characterization of *C. albicans* and *A. sclerotigenum* Crude Lipases. *3.2.1. Effect of Different pHs on Activity and Stability.* The optimal pH of microbial lipases varies depending on the species, but generally, it is near neutral. Any significant pH change in a medium results in enzyme denaturation, which lowers its activity. Enzyme activity and stability from both strains were investigated at different pHs (5.0−12) at 37 and 40 °C for *C. albicans* and *A. sclerotigenum* lipase, respectively, as shown in Figure 1. The maximum activity and stability (100%) of the *C. albicans* lipase were observed at pH 7.0, while those of the *A. sclerotigenum* lipase were observed at pH 8.0, thus confirming their optimum pH. At pH 7.0, 8.0, 9.0, and 10.0, the stabilities of *C. albicans* and *A. sclerotigenum* lipases were found to be 100, 96, 83, 58%, and 79, 100, 97, and 71%, respectively. Thus, lipases from both sources resisted the pH range from 7.0 to 10.0 and exhibited more than 50% stability. However, the stability dropped to 47% or less in the higher alkaline pH ranges of 11.0 and 12.0. Lipase activity was found to be high in the neutral to alkaline pH range. The alkaline tolerance of these lipases is appropriate for their application in laundry detergents. A number of fungal lipases are active at temperatures between 30 and 40 °C and pH levels between 6.0 and 8.0. The stability of lipases produced by *Candida* sp. at different pH ranges was also investigated in a previous study, and the results showed that the lipases remained stable within a pH range of 7.5 to 9.5.[46] Studies of *Candida rugosa* lipase stability focused mostly on the structural changes produced by pH variations. This alteration could reduce or increase pressure on the lid that covers the active core, opening or closing the catalytic site for substrate binding.[47] The activity of lipase from *Staphylococcus aureus* was determined at pH 12, commonly used in the detergent industry.[48] *Penicillium cyclopium* lipase displayed the maximum activity at pH 10, but stability decreased to around 60% of the initial level after an incubation of 120 min in pH 10 buffer.[49] Lipase derived from *Aeromonas caviae* LipT51 showed high stability and activity at elevated pH levels (6−11) and optimal activity at pH 9.0.[50] Alkaline lipases, like those discovered in this study, present a promising prospect for the biobased detergent industry.

3.2.2. Effect of Different Temperatures on Activity and Stability. Temperature has a significant effect on enzyme biocatalytic activity. Activity and stability profiles of lipases from *C. albicans* and *A. sclerotigenum* were tested at various temperatures, keeping pH 7.0 (for *C. albicans* lipase) and 8.0 (for *A. sclerotigenum* lipase) in all assays, as shown in Figure 2. The *C. albicans* lipase showed maximum activity and stability (100%) at 37 °C, while the *A. sclerotigenum* lipase showed maximum activity at 40 °C, thus confirming their optimum temperatures. *C. albicans* lipase stabilities at 30, 37, 40, 47, and 50 °C were determined to be 62, 100, 79, 69, and 61%, respectively, and *A. sclerotigenum* lipase stabilities at these temperatures were determined to be 66, 75, 100, 81, and 66%, respectively. The high thermal stability (30−50 °C) and alkaline properties (7−10) of *C. albicans* and *A. sclerotigenum* lipases suggest that they could be valuable for application in the detergent industry, thus indicating their broad and potential utility. According to a study conducted by Raza et al., the lipase produced by the strain *S. aureus* exhibited its highest activity at a temperature of 52 °C.[51] The lipase activity of *C. antarctica* was maximal at 35 °C, and maximum stability was at 30 °C.[52] *Antrodia cinnamomea* lipases demonstrated 50% enzyme residual activity in the range of 25 to 40 °C.[53] The optimal temperature for *Aspergillus tamarii* JGIF06 lipase was 37 °C,[19] while *Aspergillus melleus* showed maximum lipase activity at a temperature of 40 °C.[54] In the context of detergent formulations, it is crucial for enzymes to exhibit both activity and stability across a broad temperature range. This is particularly relevant for cleaning cotton fabrics stained with oils, as the enzymes must remain stable and active during

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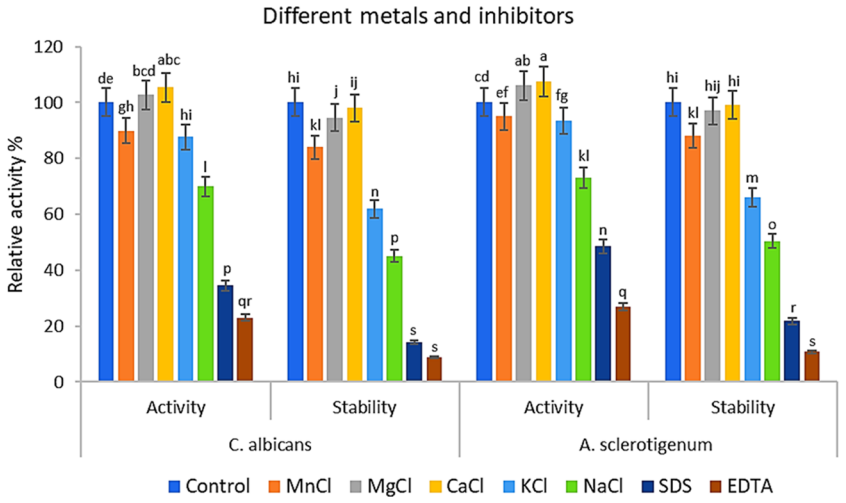

Figure 3. Effect of certain metal ions as well as potential inhibitors on the activity and stability of lipases from *C. albicans* and *A. sclerotigenum*. Activities were determined at 37 °C and pH 7.0 for *C. albicans* lipase and at 40 °C and pH 8.0 for *A. sclerotigenum* lipase using a Tween-80 substrate. Stability profiles were determined by incubating enzymes with 1 mM concentrations of metal ions and inhibitors for 2 h. The control, in which no metal ion or inhibitor was added, was assumed to be 100%. Each point in the graph shows the mean obtained from three individual experiments. The vertical bars represent the standard error of the mean. Means having different letters are statistically different ($p < 0.05$), while means sharing the same letters are statistically at par with each other.

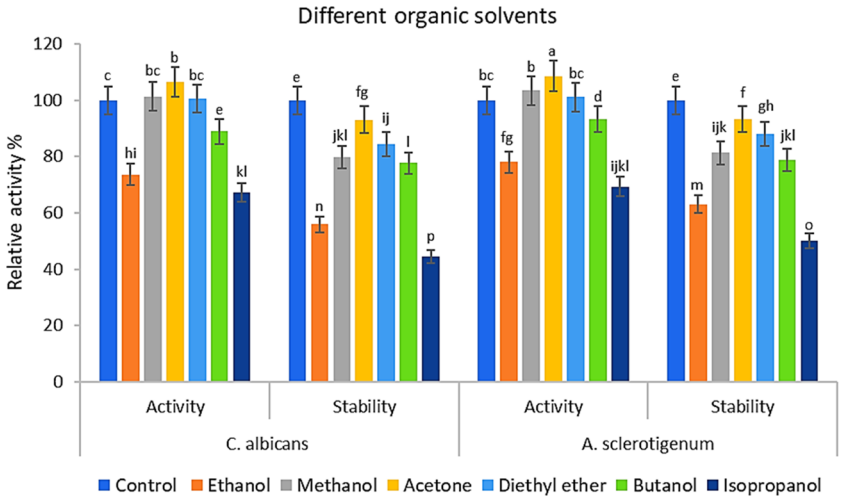

Figure 4. Effect of certain organic solvents on the activity and stability of lipases from *C. albicans* and *A. sclerotigenum*. Enzymes were incubated with organic solvents for 2 h, and activities were measured under the same conditions, using Tween-80 substrate at 37 °C, pH 7.0, and 40 °C, pH 8.0, for *C. albicans* lipase and *A. sclerotigenum* lipase, respectively. The activity level was then represented as a percentage in the absence of organic solvents. The enzymatic activity was assumed to be 100% in the absence of any organic solvent. Each point in the graph shows the mean obtained from three individual experiments. The vertical bars represent the standard error of the mean. Means having different letters are statistically different ($p < 0.05$), while means sharing the same letters are statistically at par with each other.

washing at raised temperatures.[55] Thus, the enzymes discovered in this study are significant for the detergent industry, as they showed activity at temperatures of 30−50 °C.

3.2.3. Effect of Activators and Inhibitors on Activity and Stability. The effects of various metal ions, including Mn^{2+}, Mg^{2+}, Ca^{2+}, K^{+}, and Na^{+}, and potential inhibitors SDS and EDTA, were studied in *C. albicans* and *A. sclerotigenum* lipases, as summarized in Figure 3. Enzymes showed maximum activity in the presence of MgCl and CaCl. Mg^{2+} potentiated *C. albicans* and *A. sclerotigenum* lipase stabilities up to 95 and 97%, respectively, while Ca^{2+} enhanced their stability profiles up to 98 and 99%. Na^{+} exhibited the least extent of activity and stability in lipases from both strains. SDS and EDTA severely reduced their activities. SDS decreased the stability of *C. albicans* and *A. sclerotigenum* lipases to 14 and 22%, while EDTA decreased their stability to 9 and 11%, respectively. The presence of Ca^{2+} has been shown to enhance the relative activity of the enzyme possibly due to its ability to promote a more stable conformation when bound to the active site of the enzyme. This suggests that Ca^{2+} could play a critical role in optimizing the activity and stability of the enzymes.[56] *Staphylococcus capitis* activity was increased by multiple metal ions. Among these metal ions, the maximum relative activity was observed after the treatment of the enzyme with K^{+}, Ca^{2+}, Mg^{2+}, and N^{a+}, resulting in relative activities of 104, 102, 116, and 102%, respectively. The addition of CaCl enhanced the lipase activity, suggesting that the function of the enzyme is dependent on Ca^{2+}.[57] The behavior of our enzymes in relation to calcium exhibited similarities to that of the lipase derived from *Geobacillus stearothermophilus* FMR12.[58] The addition of

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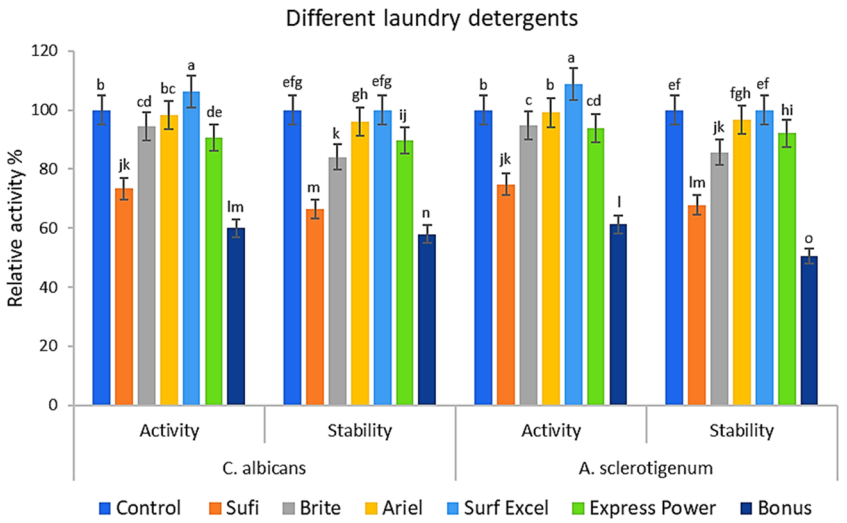

Figure 5. Effect of commercially available laundry detergents on the activity and stability of lipases from *C. albicans* and *A. sclerotigenum* after being incubated with 7 mg/mL of laundry detergents for 2 h at 37 and 40 °C, respectively. The enzymatic activity of the control, which did not include any additives and was incubated under similar circumstances, was assumed to be 100%. Each point in the graph shows the mean obtained from three individual experiments. The vertical bars represent the standard error of the mean. Means having different letters are statistically different ($p < 0.05$), while means sharing the same letters are statistically at par with each other.

SDS and EDTA to lipase led to a complete loss of enzymatic activity, attributable to the strong binding affinity of EDTA for divalent metal ions, including Ca^{2+}, which are known to bind to the active site of enzymes. EDTA can greatly diminish the catalytic activity of the lipase by sequestering the Ca^{2+} ions and causing a conformational change in enzyme structure.[59] The activity and stability of lipases may be affected differently by various metals, and even the same metal ion may behave differently when interacting with lipases from different sources.

3.2.4. Effect of Organic Solvents on Activity and Stability. The effects of different organic solvents on the activity and stability of *C. albicans* and *A. sclerotigenum* lipases were studied and are demonstrated in Figure 4. Tests conducted on various organic solvents showed that none of them had a complete inhibitory effect on the activity of lipase from either source. Lipase from both strains showed tolerance to various organic solvents (>50%). Enzyme activities were enhanced in some, i.e., acetone, methanol, and diethyl ether, while declining in others, including butanol, ethanol, and isopropanol. Maximum activities were shown with acetone (106 and 109% for *C. albicans* and *A. sclerotigenum* lipases, respectively), while minimum activities were shown with isopropyl alcohol (67 and 69%, respectively). The stability (50−93%) of both lipases was also exhibited after an incubation of 2 h. The activities declined in the following order: acetone > methanol > diethyl ether > butanol > ethanol > isopropanol, while their stability profiles decreased as follows: acetone > diethyl ether > methanol > butanol > ethanol > isopropanol. The decrease in activity of lipases when incubated with some organic solvents. This could be attributed to the dehydration action of organic solvents, which removed the water molecules from the vicinity of enzymes, precipitated them, and reduced their activity. Furthermore, any of these organic solvents could have triggered conformational changes in residues of amino acids present inside enzyme molecules. More resistance to acetone, diethyl ether, and methanol is highly advantageous for maintaining the original structure and function of enzymes in detergents. Organic solvents are commonly utilized in lipase enzymatic reactions because these solvents can assist in the handling of hydrophobic substrates such as lipids. Additionally, organic solvents have the ability to modulate and enhance the selectivity and activity of certain lipases. PCrL exhibited excellent stability in organic solvents, showing residual activity ranging from 94 to 150% following 24 h of incubation.[60] The use of enzymes, particularly lipases, in organic solvents is becoming increasingly important in industrial applications due to their potential to produce high-value products. This is because the use of enzymes in organic solvents can facilitate the synthesis of compounds that may be difficult or impossible to obtain using traditional chemical methods. Therefore, the application of lipases in organic solvent-based reaction systems holds promise for the production of valuable products.[61] Multiple factors can diminish the lipase catalytic functions in organic solvents by altering the physiology and structure of the lipases including the critical loss of water from the enzyme surface. The degree of solvent susceptibility can vary widely among lipases, with water-miscible solvents like isopropanol causing more substantial enzyme inactivation compared to water-immiscible solvents.[62] Hence, the stability of our lipases in different organic solvents makes them a potential option for implementation in detergents.

3.2.5. Effect of Laundry Detergents on Activity and Stability. Several considerations are taken into account while selecting lipases that are detergent-compatible. They are as follows: activity as well as stability; broad substrate specificity; excellent stain removal capabilities; and compatibility with diverse detergent components.[63] *C. albicans* lipase showed maximum activity in the detergent Surf Excel (106%), followed by Ariel (98%), while *A. sclerotigenum* lipase demonstrated slightly better activity in similar detergents (109 and 99%, respectively). The activity of both lipases (minimum to maximum) in the presence of all detergents was found to be as follows: Bonus < Sufi < Express Power < Brite < Ariel < Surf Excel. Lipase from both strains was found to be completely stable (>99%) after incubation for 2 h in the presence of a commercially available laundry detergent, i.e., Surf Excel. The enzymes were also stable (>50%) with other detergent brands, including Sufi, Brite, Ariel, Express Power, and Bonus (Figure

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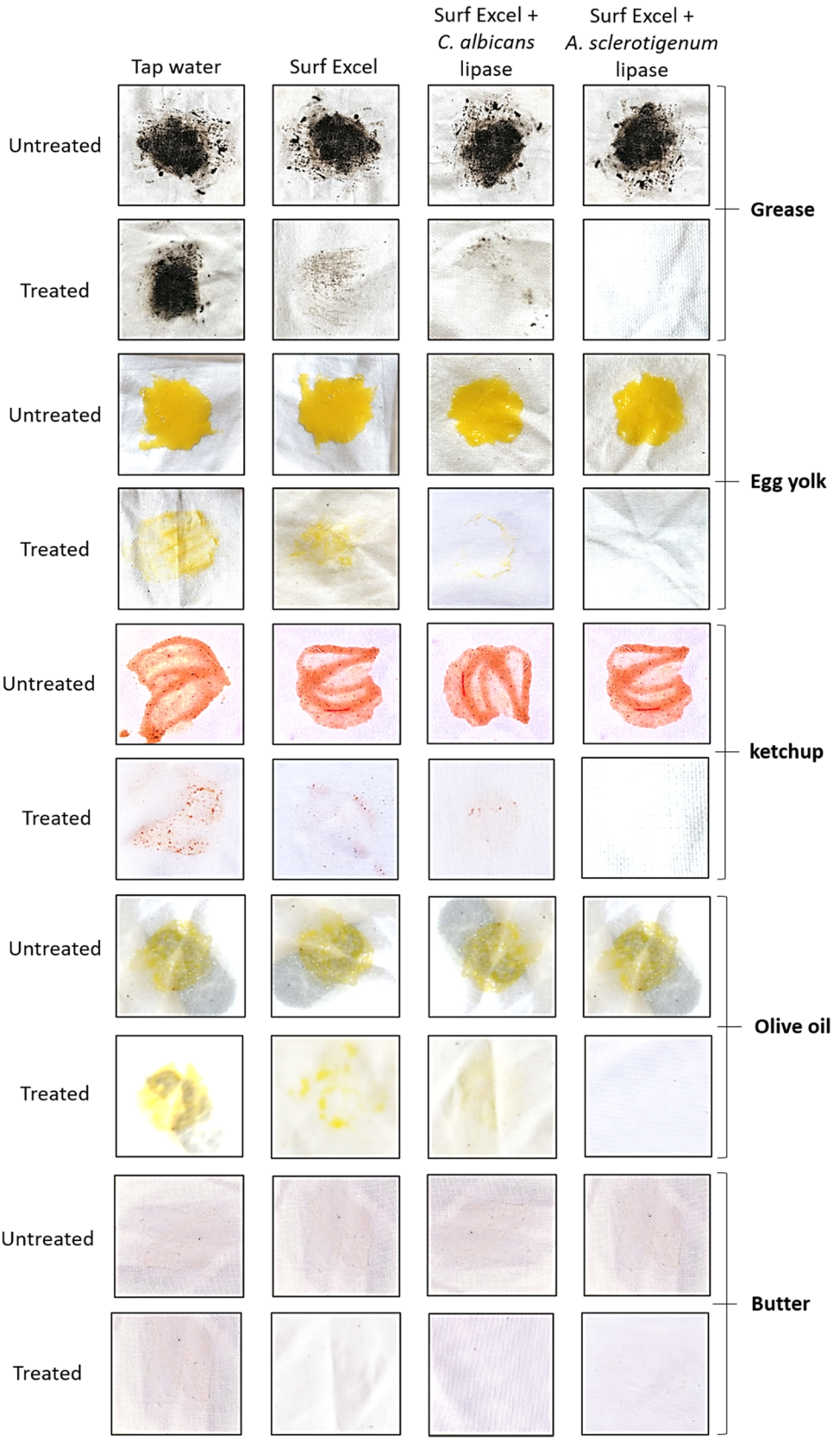

Figure 6. Stain removal performance test using a commercially available laundry detergent in the presence of lipases from *C. albicans* and *A. sclerotigenum*. This washing performance test was carried out with 7 mg/mL heat-inactivated Surf Excel detergent on cotton clothes stained with grease, egg yolk, ketchup, olive oil, and butter. The stained cloth pieces were washed with tap water, with 7 mg/mL Surf Excel, with Surf Excel supplemented with *C. albicans* lipase (51.66 U/mL), and with Surf Excel supplemented with *A. sclerotigenum* lipase (52.12 U/mL).

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ACS Omega 2023, 8, 32740−32751

5). The results showed that the lipases from both strains could survive the chemical composition of additives in detergent formulations, making them promising options for industrial-level detergent applications. *Penicillium crustosum* Thom lipase (PCrL) was identified as a novel extracellular lipase from the Ascomycota (a filamentous fungus) strain P22 and exhibited outstanding compatibility and stability with a broad range of commercially available laundry detergents.[60] Efficient functioning of lipase under harsh conditions is crucial for its effectiveness in detergent formulations. To achieve this, the stability and compatibility of the enzyme with all commercially available detergent formulations are important.[64] Characterization of lipases from psychrotrophic fungus isolates BPF4 and BPF6 revealed that they belong to the species *Penicillium canesense* and *Pseudogymnoascus roseus*, respectively. The compatibility and stability of BPF4 and BPF6 lipases were assessed in the presence of various laundry detergents. Both lipases demonstrated reasonable stability, with over 90% residual activity after 1 h of incubation in the presence of popular and commercial detergent brands.[65] The lipase from the fungus *Talaromyces thermophilus* (TTL) demonstrated excellent resistance to both interfacial denaturation and alkaline pH, and the enzyme exhibited suitability as a detergent additive. TTL showed excellent tolerance to a variety of surfactants and washing agents.[66] This feature, together with the great stability of our enzymes in a variety of detergents at alkaline pH, makes them a good choice for use in detergent compositions.

3.2.6. Wash Performance Analysis in the Presence of Crude Lipase Extracts. Lipase addition to the formulations of detergents has been studied in order to remove saucy, fatty, or greasy stains due to its usefulness during washing conditions. Lipases of both strains showed catalytic activity when tested in the presence of different detergents. *C. albicans* and *A. sclerotigenum* lipases displayed great stability in all of the tested detergents by sustaining enzyme activity, thus validating their detergent compatibility. Indeed, both lipases were shown to be extremely compatible with Surf Excel, followed by Ariel, Express Power, Brite, Sufi, and Bonus; however, *A. sclerotigenum* lipase performed slightly better. *C. albicans* and *A. sclerotigenum* lipase, when added to heat-inactivated detergent (Surf Excel), significantly removed a variety of stains, including grease, egg yolk, ketchup, olive oil, and butter stains with greasy or fat material, within 10 min. This study demonstrated that lipases derived from *C. albicans* and *A. sclerotigenum* are effective at removing stains from cloth pieces. In contrast, when cloth pieces were washed using tap water only or water containing detergent without any enzyme, incomplete removal of stains was observed, as shown in the first and second columns of Figure 6. However, the addition of lipase resulted in the almost complete removal of stains from cloth pieces, as shown in the third column (solution containing heat-inactivated detergent with lipase from *C. albicans*) and the complete removal of stains from cloth pieces, as shown in the fourth column (solution containing heat-inactivated detergent with lipase from *A. sclerotigenum*) of Figure 6. Lipase from both strains can effectively remove stains from clothes by hydrolyzing the fats or grease present in the stains; however, *A. sclerotigenum* lipase performed slightly better. Although visual examination is not sufficient for assessing wash performance, the findings of wash performance analysis demonstrated the scope and efficiency of *C. albicans* and *A. sclerotigenum* lipase in the removal of various stains.

The detergent industry highly values enzymes with broad thermostability (20−50 °C) and 6−11 pH values, which are ideal characteristics for use as additives in detergents at high pH and low temperatures.[67] In addition to their potential use in the detergent industry, lipases have numerous other biotechnological uses in various industrial sectors. These include the textile, paper, pulp, food, oleochemical, fat, and pharmaceutical industries. It is estimated that 1000 tons of lipases are added to over 13 billion tons of laundry detergent each year in the detergent industry.[68] Although visual examination is not sufficient for assessing wash performance, various forms of fatty acids, lipids, vitamins, and cholesterol are found in saucy, greasy, or oily stains, encouraging the use of lipases in the detergent industry.[69] The addition of enzymes to laundry detergent can improve washing performance and reduce the amount of detergent needed, thus providing an economic benefit by decreasing the cost and chemical burden caused by detergents.[70] Laundry detergents contain a variety of components, including bleaching agents, builders, and enzymes, which work together to prevent soil deposition onto fabrics, inhibit corrosion, add alkalinity, and maintain buffering capacity.[71] In addition to bleaching agents, builders, and enzymes, laundry detergents also contain surfactants, which reduce the surface tension at the interfaces of oil and water and make it easier to remove dirt. However, these surfactants can be toxic to aquatic life and damage the mucus layer that protects fish from bacteria and parasites. The reduced surface tension caused by surfactants in the water can also lead to the absorption of organic chemicals, pesticides, phenols, and other contaminants by aquatic organisms.[72] Surfactants in water can cause stable foam formation on the water surface, which can restrict the quantity of sunlight entering the sea or river bed because of the opaque appearance of foams with an extremely high fraction of liquid and have an adverse effect on plant photosynthesis.[73] In these circumstances, using less detergent and fewer chemicals is important for the environment and can be accomplished by adding eco-friendly and completely biodegradable enzymes to detergents as additives. Enzyme-based laundry detergents require lower amounts to achieve excellent washing performance, are less hazardous to the environment, and have less of an impact on the color and quality of fabrics in laundry compared with synthetic detergents. All of these results indicate that *C. albicans* and *A. sclerotigenum* lipases are suitable for use in the detergent industry.

4. CONCLUSIONS

In the present study, two different strains (*C. albicans* and *A. sclerotigenum*) produced thermostable and alkaline lipases using wheat bran as the primary substrate under solid-state fermentation. Both lipases displayed outstanding activity and stability, particularly over a wide range of pH and temperature levels, with optimal activity at 37 °C and pH 7.0 for *C. albicans* and 40 °C and pH 8.0 for *A. sclerotigenum*, respectively. The results showed that these enzymes were extremely stable after treatment with different metal ions, organic solvents, and commercially available detergents. Importantly, they improved the ability to remove stains from cotton clothes, suggesting them as efficient bioadditives in detergent formulations. The enormous potential of these lipases in removing various stains following their activity and stability characterization proved helpful in the development of an easy, efficient, and eco-friendly approach for the productivity and applicability of these

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valuable enzymes in the detergent industry. To improve the lipase yield from *C. albicans* and *A. sclerotigenum*, future research should focus on investigating gene recombination and overexpression techniques. Furthermore, other innovative molecular-level approaches could be employed for increased lipase production in order to meet the demands of the industrial sector.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03644.

Lipase production (Table S1) and lipase production from *C. albicans* and *A. sclerotinum* confirmed by a primary plate screening assay using methyl red indicator and Tween-80 substrate (Figure S1) (PDF)

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