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Comparative Screening of Fungal Isolates for Dextranase, Glucoamylase, and Carboxymethyl Cellulase Production

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ABSTRACT

Polysaccharide-hydrolyzing enzymes are important biocatalysts in biomass conversion, food and sugar processing, fermentation, and agro-waste valorization. Soil samples collected at a depth of 20 cm from geographically separated sites in five Egyptian governorates were cultured on potato dextrose agar (PDA), and 15 fungal isolates (F1–F15) were recovered. Each isolate was screened for dextranase, glucoamylase, and carboxymethyl cellulase (CMCase) production under submerged fermentation. Reducing sugars were quantified by the dinitrosalicylic acid method. The isolates showed distinct and complementary enzyme-production profiles. F11 produced the highest dextranase-associated release (0.1156 mg/mL), F8 was the leading glucoamylase isolate (0.8528 mg/mL), and F6 showed the highest CMCase-associated release (0.1977 mg/mL). Several isolates expressed more than one detectable activity, supporting their potential use in enzyme combinations and biomass-processing applications.

Keywords: soil fungi, dextranase, glucoamylase, CMCase, medical entomology

1. Introduction

The enzymatic depolymerization of complex carbohydrates is central to sustainable industrial biotechnology. Dextranase (EC 3.2.1.11) hydrolyses dextran and is useful in sugar processing; glucoamylase (EC 3.2.1.3) releases glucose from starch; and endo-β-1,4-glucanase activity measured with carboxymethyl cellulose (CMCase; EC 3.2.1.4) contributes to cellulose conversion (Farmani *et al.*, 2022; Kumar and Satyanarayana, 2009; Kubicek *et al*., 2009). Filamentous fungi are attractive producers because they secrete extracellular proteins efficiently, although productivity can vary markedly among isolates and with medium composition, morphology, and culture conditions (Papagianni, 2004).

Comparative enzyme screening requires a response variable that corresponds directly to the analytical method. The dinitro salicylic acid (DNS) assay estimates reducing sugars (Miller, 1959); accordingly, the present study reports paired-background-corrected glucose-equivalent reducing-sugar release for each isolate.

The study also has a relevant connection to medical entomology. Enzymatic saccharification of cellulose- and starch-rich residues can generate low-cost carbon sources for fermentation of microbial control agents. Sugarcane bagasse, for example, has been investigated as a substrate for production of

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Bacillus thuringiensis (Bt), a bacterial larvicide (Borin *et al*., 2015; Poopathi *et al*., 2013). Enzyme-assisted pretreatment could therefore improve the efficiency, cost, and consistency of such production processes.

A second prospective route concerns polysaccharide-rich matrices in mosquito habitats. Biofilms accumulate in mosquito-rearing and breeding containers, and breeding-site microbial communities interact with oviposition and larval development (Hunt *et al*., 2020; Mosquera *et al*., 2023). Dextranase or CMCase could be evaluated against chemically characterized extracellular polymeric substances, but the biological effect is not predictable: hydrolysis might disrupt a matrix or, conversely, release soluble sugars that nourish microbes and larvae. This bi-directionality makes controlled testing essential.

Fungal enzymes also have wider relevance to biological control. The extracellular enzymes most closely associated with penetration of insect cuticle include chitinases, proteases, and lipases (da Silveira *et al*., 2021; Dhawan and Joshi, 2017; Gebremariam *et al*., 2022), while fungal metabolites and chitinolytic complexes have demonstrated mosquito-larval activity (Ragavendran *et al*., 2019; Banduwardena *et al*., 2025; da Silveira *et al*., 2021). Screening environmental fungal isolates for complementary enzyme activities can therefore support programmes that combine producer selection, enzyme characterization, biomass valorization, and biological-control product development.

The primary objective was to screen 15 fungal isolates for production of dextranase, glucoamylase, and CMCase and to identify the most promising producers for subsequent characterization and optimization. A secondary objective was to explore the wider biotechnological value of the resulting isolate matrix, including industrial processing, agro-waste valorization, fermentation-feedstock development, and applications relevant to medical entomology.

2. Materials and Methods

2.1 Sampling locations

Soil samples were collected aseptically at a depth of 20 cm from selected sites in five Egyptian governorates: Menoufia, Sharqia, Gharbia, Fayoum, and Qena. Sampling was distributed across geographically separated areas representing the Nile Delta, the Fayoum region, and Upper Egypt to broaden the environmental coverage of the culture-based screening. The samples were transferred to the laboratory in sterile containers and used for fungal isolation (Afify *et al*., 2009).

2.2 Isolation and purification of fungal isolates

Fungal isolation followed a soil-suspension approach based on Afify *et al*. (2009), modified for fungal recovery. Potato dextrose agar (PDA) served as both the primary isolation and purification medium. Approximately 10 g of each muddy soil sample was suspended in 50 mL sterile distilled water and shaken thoroughly. Aliquots of the resulting suspension were used to inoculate PDA plates, which were incubated at 28°C until fungal colonies developed. Morphologically distinct colonies were picked and purified by repeated subculturing on fresh PDA. Fifteen purified fungal isolates were recovered, coded F1–F15, and maintained on PDA slants until enzyme screening.

2.3 Submerged fermentation and crude enzyme preparation

Each of the 15 fungal isolates was cultivated separately under submerged-fermentation conditions for enzyme production. Cultivation used 250-mL Erlenmeyer flasks containing 50 mL minimal salts medium: KH_2PO_4, 2.0 g/L; $MgSO_4 \cdot 7H_2O$, 0.3 g/L; $CaCl_2$, 0.3 g/L; and yeast extract, 1.0 g/L. The initial pH was adjusted to 5.5. The relevant carbon inducer was supplied at 1% (w/v): dextran for dextranase, soluble starch for glucoamylase, or low-viscosity carboxymethyl cellulose for CMCase. Cultures were incubated at 28°C and 150 rpm for 96 h. Following incubation, cultures were centrifuged at 10,000×g for 15 min at 4°C, and the cell-free supernatants were collected as crude enzyme preparations.

2.4 Enzyme assays

Reducing sugars were determined by the DNS method (Miller, 1959). Each isolate was examined in the three substrate-specific assays, and technical absorbance readings were recorded in duplicate where available. A control absorbance was used to correct the background of each crude preparation.

Dextranase reactions contained 0.5 mL crude enzyme supernatant and 0.5 mL of 1% dextran in 0.05 M sodium acetate buffer (pH 5.0) and were incubated at 40°C for 15 min. Glucoamylase reactions contained 0.5 mL crude enzyme supernatant and 0.5 mL of 1% soluble starch in 0.05 M citrate buffer (pH 4.5) and were incubated at 50°C for 15 min. CMCase reactions contained 0.5 mL crude enzyme supernatant and 0.5 mL of 1% carboxymethyl cellulose in 0.05 M sodium citrate buffer (pH 4.8) and were incubated at 50°C for 30 min.

Reactions were stopped and colour developed by adding 1.0 mL DNS reagent and heating in a boiling-water bath for 5 min. After cooling to room temperature, absorbance was read at 550 nm. The mean sample absorbance was compared with its control absorbance.

2.5. Calibration and calculation of enzyme-associated sugar release

Two glucose-equivalent calibration datasets were present in the source workbook: a 0.1–1.0 mg/mL dextrose series used for dextranase and CMCase, and a 0.1–0.5 mg/mL glucose series used for glucoamylase. Ordinary least-squares regression with an intercept was fitted to the mean duplicate absorbance at each standard concentration.

For each isolate, net release was calculated from the difference between the mean sample absorbance and its control absorbance, divided by the relevant calibration slope. The resulting values are reported as mg/mL glucose-equivalent reducing-sugar release.

The dextrose calibration used for the dextranase and CMCase assays covered 0.1–1.0 mg/mL and yielded the regression equation Abs = 2.0590C + 0.1634 (R^2 = 0.9914). The glucose calibration used for the glucoamylase assay covered 0.1–0.5 mg/mL and yielded the regression equation Abs = 0.0985C + 0.09715 (R^2 = 0.9537). Because this curve showed lower linearity and some sample absorbances exceeded its upper standard, the glucoamylase values were interpreted as preliminary screening estimates; subsequent quantitative optimization should use sample dilution and a calibration range that brackets all sample absorbances.

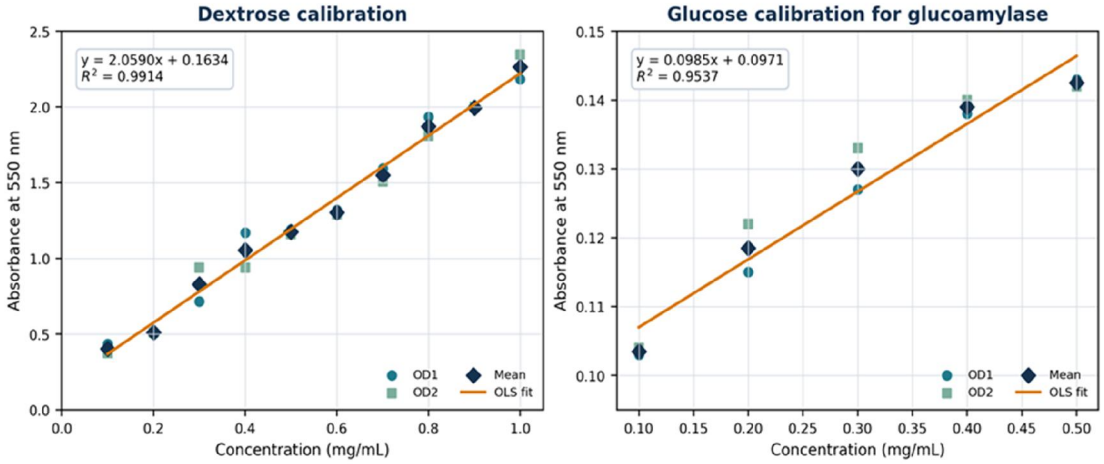

Fig. 1: Corrected calibration curves. The dextrose curve was used for dextranase and CMCase; the lower-range glucose curve was used for glucoamylase. The underlying values and native editable charts are supplied in Supplementary File S1.

2.6. Data handling and descriptive analysis

Technical readings were averaged to obtain one net value for each isolate in each enzyme assay. Results were summarized descriptively, and the 15 isolates were ranked according to net glucose-equivalent reducing-sugar release. The complete calculations and editable charts are provided in Supplementary File S1.

2.7. Calibration performance and data verification

During verification of the dextrose standards, the OD1 entry at 0.5 mg/mL was corrected from 0.911 to 1.191 as the internally consistent decimal entry; both the original and corrected values are

retained in Supplementary File S1. Negative blank-corrected readings were recorded as zero. The resulting dextrose calibration was linear over 0.1–1.0 mg/mL (Abs=2.0590C+0.1634, R^2=0.9914). The glucoamylase glucose calibration was approximately linear over 0.1–0.5 mg/mL (Abs=0.0985C+0.09715, R^2=0.9537). The raw readings, formula-linked calculations, and editable curves are provided in Supplementary File S1.

3. Results

3.1. Dextranase screening

Dextranase activity varied markedly among the 15 isolates. F11 produced the highest net value (0.1156 mg/mL), followed by F9 (0.0777 mg/mL), F13 (0.0593 mg/mL), F12 (0.0471 mg/mL), and F8 (0.0459 mg/mL). Lower positive values were recorded for F1, F3, F4, F5, and F7, whereas F2, F6, F10, F14, and F15 showed no net release above their control, , as reported in Table (1) and showed in Figure (2).

3.2. Glucoamylase screening

Table (1) and Figure (2) reported that, glucoamylase activity was highest in F8 (0.8528 mg/mL), followed by F2 (0.3858 mg/mL). Moderate values were recorded for F7 (0.1472 mg/mL), F3 (0.1421 mg/mL), and F4 (0.1218 mg/mL), while F6 produced a low positive value (0.0152 mg/mL). The remaining isolates showed no net glucoamylase-associated release above control.

3.3. CMCase screening

CMCase activity was concentrated in three isolates. F6 produced the highest value (0.1977 mg/mL), followed by F5 (0.1323 mg/mL) and F4 (0.0954 mg/mL). The other 12 isolates showed no net CMCase activity above their control samples under the tested conditions, as reported in Table (1) and showed in Figure (2).

Table 1: Net glucose-equivalent reducing-sugar release of the 15 soil fungal isolates in the three enzyme assays.

Isolate	Dextranase (mg/mL)	Glucoamylase (mg/mL)	CMCase (mg/mL)	Detected profile
F1	0.0299	0.0000	0.0000	Dextranase
F2	0.0000	0.3858	0.0000	Glucoamylase
F3	0.0138	0.1421	0.0000	Dextranase + Glucoamylase
F4	0.0019	0.1218	0.0954	Dextranase + Glucoamylase + CMCase
F5	0.0342	0.0000	0.1323	Dextranase + CMCase
F6	0.0000	0.0152	0.1977	Glucoamylase + CMCase
F7	0.0267	0.1472	0.0000	Dextranase + Glucoamylase
F8	0.0459	0.8528	0.0000	Dextranase + Glucoamylase
F9	0.0777	0.0000	0.0000	Dextranase
F10	0.0000	0.0000	0.0000	No net release
F11	0.1156	0.0000	0.0000	Dextranase
F12	0.0471	0.0000	0.0000	Dextranase
F13	0.0593	0.0000	0.0000	Dextranase
F14	0.0000	0.0000	0.0000	No net release
F15	0.0000	0.0000	0.0000	No net release

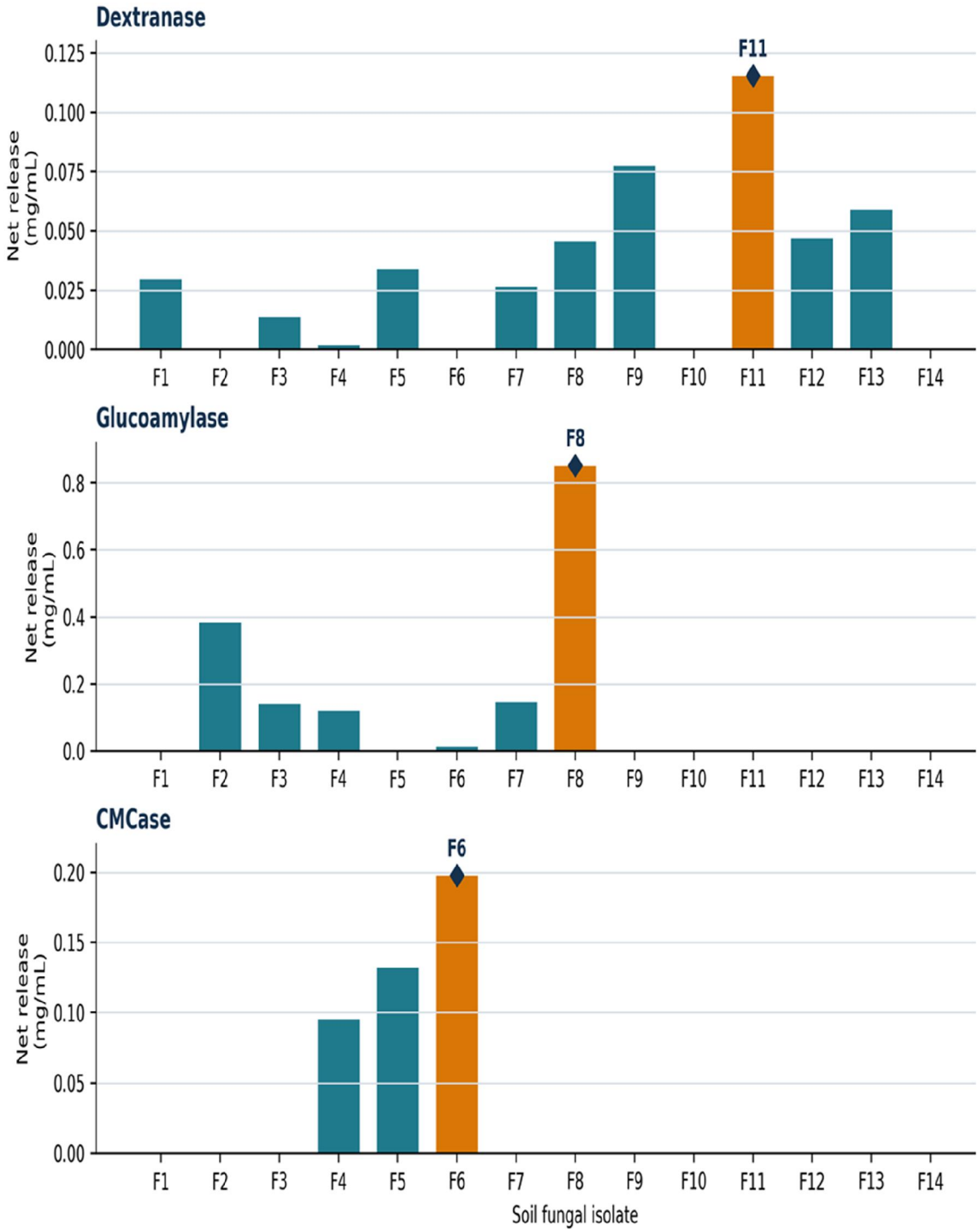

Fig. 2: Enzyme-production profiles of the 15 soil fungal isolates. Bars represent the net glucose-equivalent reducing-sugar release calculated from the mean technical absorbance readings. The diamond identifies the highest-value isolate in each assay.

3.4. Producer-selection interpretation

The isolate-level profiles identified one leading candidate for each enzyme: F11 for dextranase, F8 for glucoamylase, and F6 for CMCase. F4 showed detectable release in all three assays, while F3,

F5, F6, F7, and F8 expressed combinations of two activities. These isolates provide a focused group for taxonomic identification, biological replication, enzyme characterization, and fermentation optimization.

4. Discussion

The 15 soil fungal isolates displayed heterogeneous and complementary enzyme profiles across the three enzyme substrates. Dextranase activity was distributed across several isolates, glucoamylase was dominated by F8 and F2, and CMCase was concentrated in F4–F6. This diversity is valuable for selecting individual producers or designing defined enzyme combinations for biomass processing.

F11, F8, and F6 were the leading descriptive candidates for dextranase, glucoamylase, and CMCase, respectively. F4 is also noteworthy because it produced detectable release in all three assays, suggesting a broader hydrolytic profile. Enzyme expression in filamentous fungi is influenced by inducer identity, carbon catabolite repression, medium composition, pH, morphology, oxygen transfer, and fermentation time (Kubicek *et al*., 2009; Papagianni, 2004; Singhania *et al.,* 2010; Peterson and Nevalainen, 2012; Nevalainen and Peterson, 2014). Consequently, optimization may increase yields or reveal additional activities in the lower-producing isolates.

The wide range of isolate-level values provides an efficient first screening step. The highest-producing isolates should next be evaluated in independent biological cultures and identified by morphological characterization and ITS rDNA sequencing. This will establish production reproducibility, support taxonomic assignment, and inform the commercial and biosafety assessment of F6, F8, and F11.

The reported response was aligned with the analytical method: DNS absorbance and glucose-equivalent calibration were used to quantify reducing-sugar release. Expressing the screening values as net glucose-equivalent release provides a consistent basis for ranking isolates. Subsequent optimization can incorporate substrate-specific activity, dilution factors, reaction time, enzyme volume, and protein-normalized activity.

The variation among isolates emphasizes the importance of standardizing inoculum quality, fungal morphology, oxygen transfer, incubation conditions, and harvest time. Extending the calibration range and applying sample dilution where necessary would further improve quantitative precision in subsequent optimization studies.

A promising link to vector control is upstream bioprocessing. CMCase from F6 can be evaluated for saccharification of cellulose-rich residues such as bagasse; F8 glucoamylase can be examined with starch-rich by-products; and F11 dextranase can be assessed with dextran-bearing sugar streams. The resulting hydrolysates could serve as defined carbon sources for *Bt* or another microbial-control production process (Borin *et al*., 2015; Poopathi *et al*., 2013).

This route may be especially attractive where agro-industrial residues are locally abundant and the cost of conventional media limits decentralized bio larvicide production. However, hydrolysate composition, inhibitor formation, batch variability, sterilization needs, downstream potency, shelf life, and quality control must be measured before any economic or public-health claim.

Mosquito-breeding containers can develop biofilms, and Aedes egg-laying can shape bacterial communities in breeding sites (Hunt *et al*., 2020; Mosquera *et al*., 2023). Defined dextranase or CMCase preparations could be used as research probes if dextran- or cellulose-like components are chemically demonstrated in the extracellular matrix. This is an exploratory concept rather than a recommended field treatment. Hydrolysis could reduce matrix integrity, but released oligosaccharides could also enhance microbial growth or larval nutrition. Oviposition, larval development, microbial composition, dissolved organic carbon, and residual activity would all need simultaneous measurement.

Cell-free fungal preparations and fungal isolates have produced larval effects in other studies (Ragavendran *et al*., 2019; Banduwardena *et al*., 2025), and a chitinolytic complex from *T. asperellum* damaged *Aedes aegypti* larvae (da Silveira *et al*., 2021). These findings highlight the value of evaluating prioritized environmental isolates for complementary chitinases, proteases, and lipases, whose production can correlate with fungal virulence (Dhawan and Joshi, 2017; Gebremariam *et al*., 2022). Combining those enzymes with the polysaccharide-hydrolase profiles identified here may support new formulations and integrated biological-control strategies.

Further development can compare active filtrates, heat-inactivated preparations, enzyme-depleted fractions, purified enzymes, and appropriate controls to distinguish enzyme-associated effects from those of secondary metabolites. Evaluation in insecticide-susceptible and resistant mosquito populations would also clarify the value of optimized fungal products within resistance-management programmes.

Selection by enzyme yield should be followed by taxonomic identification, metabolite profiling, and biosafety assessment of the prioritized isolates. A translational pathway can prioritize purified enzymes, cell-free products, or characterized hydrolysates and should include contaminant and toxin screening, non-target testing, production containment, and regulatory review, as suggested in Figure (3).

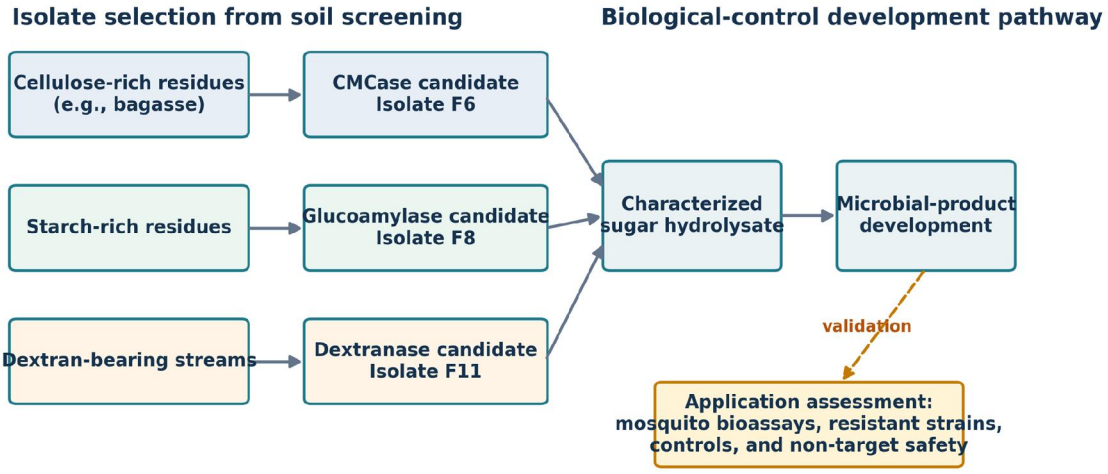

Fig. 3: Potential pathway for integrating the leading soil fungal isolates into agro-residue valorization, microbial-product development, and biological-control assessment.

5. Recommendations

1. Repeat the leading isolates in at least five independent biological cultures using randomized flask positions, predefined quality criteria, and standards spanning all sample absorbances.
2. Identify the prioritized isolates taxonomically using morphological characterization supported by molecular sequencing, particularly F11, F8, F6, and the multi-enzyme isolate F4.
3. Optimize each candidate separately using a formal design of experiments for pH, temperature, inducer concentration, nitrogen source, agitation, inoculum, and harvest time (Montgomery, 2017; Singhania *et al*., 2010).
4. Report substrate-specific activity in U/mL and U/mg protein after recording reaction volume, incubation time, dilution factors, glucose-equivalent stoichiometry, and total protein. Total protein should be quantified using a validated method such as the Bradford or Lowry assay.
5. Evaluate F6 CMCase for cellulose-rich residues, F8 glucoamylase for starch-rich by-products, and F11 dextranase for dextran-bearing streams; compare single-enzyme and defined multi-enzyme preparations.
6. Test characterized hydrolysates as fermentation feedstocks for *Bt* or another microbial larvicide and quantify the potency and stability of the resulting product using standardized mosquito-bioassay methods.
7. Expand screening of prioritized isolates to chitinases, proteases, lipases, and relevant enzyme combinations, with appropriate active, inactivated, and medium controls (da Silveira *et al*., 2021; Dhawan and Joshi, 2017; Gebremariam *et al*., 2022).
8. Characterize breeding-site extracellular polymeric substances before testing dextranase or CMCase as biofilm-modifying agents, and monitor both disruption and possible sugar-driven microbial stimulation.

6. Conclusion

The 15 soil fungal isolates showed complementary enzyme-production profiles. F11 was the leading dextranase candidate, F8 produced the highest glucoamylase-associated release, and F6 was the strongest CMCase producer. F4 showed detectable activity in all three assays and may be valuable for multi-enzyme development. These results establish a practical shortlist for taxonomic identification, biological replication, enzyme characterization, and fermentation optimization.

The isolate matrix has value across several biotechnological areas, including sugar and starch processing, biomass conversion, agro-waste valorization, and fermentation-feedstock development. In medical entomology, selected hydrolases can be integrated into low-cost microbial-product development, biofilm-related investigations, and controlled evaluation of biological-control preparations.

Declarations

Author contributions: All authors contributed in all parts of the manuscript.

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Conflict of interest: The authors declare no conflict of interest.

Ethics statement: Not applicable; the study used in-vitro fungal cultures and cell-free enzyme preparations.

Data availability: All processed data are presented in the manuscript; raw absorbance data are available from the corresponding author upon reasonable request.

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