Screening for the economic production of hydrolytic enzymes from locally-isolated fungi

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Background
Enzymes are complex proteins serving as biological catalysts to facilitate reactions in mild and environment-friendly conditions. Saprophytic fungi have long been harnessed for the efficient production of several industrially-significant enzymes whose market is still growing to cope with the increase in demand and natural resources' depletion.
Objective
This investigation was performed with respect to the economic viewpoint of terrestrial fungi utilization and their hydrolytic enzymes' biosynthetic potential.
Materials and methods
Several terrestrial fungi were isolated, cultivated on cheap agricultural wastes, and evaluated for industrial relevance. Solid-state fermentation was conducted to further boost the economic value and sustainability. The enzymatic productivity was estimated through solid-phase radial diffusion correlating the zones' diameters to the enzymatic activity.
Results and conclusion
Six soil fungi were isolated, five belonging to the order Eurotiales and one to Mucorales. The molds belonged to four different genera; *Aspergillus sydowii*, *Aspergillus versicolor*, *Aspergillus ustus*, *Fennelia flavipes* (anamorph: *Aspergillus flavipes*), *Cunninghamella elegans* and *Paecilomyces lilacinus*. Many of the tested agricultural wastes were able to support the biosynthesis of the explored constitutive enzymes, recording better activity than the standard synthetic medium. Under the test conditions, L-asparaginase and protease were the most frequently detected enzymes while banana and mandarine peels led to the highest enzymes' activity.
In light of the global direction towards sustainability, enzymes can have immense prospects to sustain the industrial sectors innocuously. The cost-effectiveness of the manufacturing processes can be enhanced by accommodating the fiscal challenges for operating conditions. Using agrarian residues as raw material, highly productive enzyme producers, and cheaper solid-state fermentation processes are factors that may contribute to the efficacy, efficiency and economic feasibility of the enzyme-based processes.

Keywords:
agro-industrial waste, cellulase, esterase, L-asparaginase, lipase, protease, solid-state fermentation (SSF), sustainability, urease, α-amylase

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Introduction

Enzymes are ubiquitous proteins that catalyze various chemical reactions in all forms of living organisms and have long been exploited in industrial processes since prehistoric times e.g.: baking, biofuel production, brewing, pharmaceuticals, textiles, detergents, paper industries, etc. In comparison to conventional chemical processes, they bear the advantages of reduced environmental footprints, eco-cleanliness, lower energy consumption, reaction specificity, minimal byproducts, and easier product purification [1,2]. Therefore, there is always an ongoing quest for enzymes with the promise of obtaining novel, favorable catalytic characteristics and/or easy, cheap acquisition and scalability.

Fungal enzymes, in particular, are characterized by (i) high yield, titer, and productivity, (ii) easy and inexpensive downstreaming, (iii) broad substrate promiscuity, (iv) maintained activity under rough operational conditions e.g.: broad temperature and pH ranges and minimal water activity (a_w), and (v) generally regarded as safe (GRAS) status. Therefore, they contribute more than half the enzymes employed in industry esp. from the genera *Aspergillus*, *Penicillium,* and *Trichoderma* [1,3–5]. Many enzymes are applied in

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different industries however, the biggest market participation is attributed to the Class III hydrolases, marking almost 85% of the market share while other classes are represented by smaller contributions [3]. In this regard, different enzymes were estimated in this report namely; amylase, asparaginase, cellulase, esterase, lipase, protease and urease.

Starch-digesting enzymes are α-amylases (EC 3.2.1.1) that randomly break α-1,4 glycosidic linkages or β-amylases (EC 3.2.1.2) that digest every alternate α-1,4 bonds and invert the anomeric configuration of the released maltose into the β-form [4,6]. Amylases are mainly inducible enzymes that are liable to catabolite repression upon starch degradation products presence (e.g.: maltose and glucose) while a minority of constitutive amylases were also reported [6]. It has versatile applications in brewing, detergents, fuel alcohol, food, paper and textile industries [6].

L-asparaginase (EC 3.5.1.1) irreversibly deaminates the amino acid L-asparagine into L-aspartate and ammonia. Both inductive and constitutive examples were described. It is extracellularly secreted in plants, animals, and microbes but humans are not capable of producing it [7,8]. Bacterial asparginases, unlike fungal ones, have been associated with allergic reactions that may mount up to anaphylaxis in humans. Hence, asparaginases of fungal origin are sought in applications related to human intake. It is used in acute lymphoblastic leukemia chemotherapy and food industry [8].

Cellulase (EC 3.2.1.4) is a constitutive enzyme that is upregulated in the presence of substrates [9]. It has a wide range of applications e.g.: brewing, food, textile, pulp and paper, agriculture and biofuel.

Esterases (EC 3.1.1.1) and lipases (EC 3.1.1.3) are ubiquitously secreted to hydrolyze triglycerides; simple short chain triglycerides (not more than C6) in case of esterases and longer, water insoluble, heavily aggregated fatty acids in case of lipases. They are biosynthesized constitutively, independent from lipids presence [10,11]. They are used in several sectors including, but not limited to, the food, biodiesel, detergents and pharmaceutical industries.

Proteases (EC 3.4) are ubiquitous and include a large group of enzymes that cleave the proteins' peptide bonds. They are naturally secreted constitutively or partially inducible in the respective organism. They have been applied in detergents, leather, pharmaceutical, waste and food industries representing more than 60% of the world's total industrially-employed enzymes [12].

Ureases (EC 3.5.1.5) catalyze the degradation of urea into ammonia and carbamate enabling organisms to use urea as a nitrogen source. Both constitutive and inducible producers were described [13]. Its main application is in the fields of insecticides manufacture to protect crops, treatment of industrial wastewater and analysis of urea, arginine and heavy metal content in different samples [13].

To meet the continuing increase in enzymes' demand, the production and harvesting processes must accord with economic, safety and quality parameters. Accordingly, using agricultural, industrial and environmental waste residues has risen as a pivotal factor in cost-effective manufacture, especially that fungi are classified as heterotrophs and natural decomposers (mostly saprophytes). Hence, they are capable of secreting a consortia of hydrolytic and oxidative exoenzymes through efflux pumps to efficiently digest complex substrates in the surrounding niches or as virulence factors to eradicate competing organisms [2,3]. As per sustainable and economic production considerations, agrarian lignocellulosic waste biomass (e.g.: rice husk, wheat bran, orange peel, copra meal, etc.) imposes itself as an ideal candidate on account of: (i) abundance, (ii) avoid the food versus fuel dilemma, (iii) avoid the pollution caused by leaching into fields or biomass incineration, and (iv) recovery of rich nutrients otherwise completely lost [14,15].

Furthermore, solid-state fermentation (SSF) is a low-cost strategy that has long been implemented in Asian countries and has recently gained attention in microorganisms cultivation and bioactive metabolites (e.g.: enzymes, organic acids, pigments, etc.) production, especially from fungi that grow well in xerophilic conditions [3,15]. This is explained by the resemblance of the solid, semi-solid support or substrate in the near absence of free water to the natural habitat in which the fungi normally exist thus, they adapt fast and grow decently in SSF [15,16]. Unlike submerged fermentation (SmF), it requires less energy, lower capital investment, subjects the molds to lower incidences of bacterial contamination, catabolic repression and substrate hampering, less waste water, easier product harvesting, minimal effluent processing and less labor intensive [2,15]. The use of agro-residues and forest remains, that are rich in cellulose,

hemicellulose, fibers, lignin, pectin, starch and small sugar molecules, is much exercised in SSF rather than utilizing standard defined media. This can be attributed to (i) the aptitude of saprophytes adaptation and thriving thereon, (ii) the ease of controlling the particle size and surface area via chopping, grinding and cutting, and (iii) their serving as both substrates and support material without the need for intensive pre-treatment [15,17,18].

This study was carried out to ascertain the ability of locally-isolated terrestrial soil fungi to produce industrially-significant enzymes. Furthermore, the economic production using agricultural debris, as substrates, was confirmed as a means to sustainably exploit the waste biomass in different industries.

Materials and methods

Chemicals and food waste

Chemicals were of analytical grade and purchased from Sigma-Aldrich, unless otherwise stated, and were used as received without further purification. All solutions were prepared using deionized water of resistivity not less than 18.2 MΩ.cm.

Food waste (banana, *Musa paradisiaca*; mandarine, *Citrus reticulate*; melon, *Cucumis melo*; watermelon, *Citrullus lanatus*) were obtained from local fruits vendors, cleaned and peeled. Sugarcane bagasse (*Saccharum officinarum*) was obtained from local juice extraction shop. The fruit peels and bagasse were coarsely grounded using a conventional blender, the powders were dried in an oven at 50°C until constant weight and screened through 20 mesh size.

Microorganisms and maintenance

Soil sample was collected from the top 10 to 20 cm of the surface from the National Research Centre (NRC, Giza, Egypt) herbarium (30°02′10.4369′, 031°12′18.5445′) in January 2022. 10 g of the soil sample were suspended in 50 mL sterile water and ten-fold serial dilutions were prepared from the suspension. Isolation was carried out from suitable dilution of the soil samples by spreading over the surface of agar plates of potato dextrose (PDA) and Czapek's media (CZA). After incubation for 7 days at 30°C, the plates were checked for the growth of colonies, and single colonies were selected and streaked onto the surface of agar plate of the same medium and allowed to grow for another 7 days. A touch of the terminal colonial growth of a single separate colony was transferred to pure slants of PDA medium to be preserved in 4°C and subjected to regular sub-culturing every 2 months [19]. Isolates were preliminary identified using the morphologic and microscopic features following the classic and standard description [20,21] using Olympus CX41 Microscope (Olympus Corp., Japan).

Media preparation, inoculum preparation and culture conditions

Grounded food waste, 5 g, moistened with distilled water to 50% moisture level, were sterilized and used as production medium in 250 ml Erlenmeyer flask.

For fungal isolation PDA and CZA media were used. PDA media composition (L^{-1}): peeled potato slices, 250 g; glucose, 20 g; agar, 20 g. CZA media composition (L^{-1}): sucrose, 30 g; $NaNO_3$, 2 g; K_2HPO_4, 1 g; $MgSO_4$, 0.5 g; KCl, 0.5 g; $FeSO_4$, 0.01 g; agar, 15 g.

Minimal medium was prepared as follows (L^{-1}): sucrose, 30 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4.7H_2O$, 10 mg; $NaNO_3$, 2 g; trace elements solution, 2 ml; agar, 5 g. Trace elements solution composition was (g% w/v): citric acid, 5; $ZnSO_4.7H_2O$, 5; $Fe(NH_4)_2(SO_4).6H_2O$, 1; $CuSO_4$, 0.25; $MnSO_4.H_2O$, 0.05; H_3BO_3, 0.05, $NaMoO_4.2H_2O$, 0.05 (adapted from: [41]). The moisture content was adjusted at 50%.

For inoculum preparation, 7 days old fungi slants were used to prepare a 10^7 CFU/mL spore suspension and used to inoculate the production medium at a concentration of 10^5 CFU/g dry substrate.

Cultures were then incubated at 30°C for 7 days in humidity-controlled incubator. At the end of cultivation, the content of each flask was extracted with potassium phosphate buffer (pH 7) by shaking for 1 h at 30°C followed by filtration and the supernatant was tested for various enzymatic activities [22].

Screening and evaluation of enzymatic activities

Solid-phase agar plate assays were adopted for enzymatic activities' screening and evaluation. All experiments were repeated thrice and the data is presented as means ± standard error of means.

Amylolytic activity: 0.5 g% starch in agar plates were prepared and 0.9 cm wells were cut using cork borer. Supernatant of the fungal cultures were placed in the wells and incubated for 24 h and later, thoroughly flooded with 1% Lugol's solution for 20 min. The plates were observed for the formation of a clear halo

zone against a blue-black background, indicating α-amylase production [4].

L-asparaginase activity: Agar plates with 40 mM asparagine and phenol red indicator in 50 mM Tris-HCl buffer (pH 8.6) were used. Plates were incubated and studied for red color zones indicating positive asparaginase activity [7,23].

Cellulolytic activity: Agar plates containing 1 g% w/v of sodium carboxymethyl cellulose (Na CMC) were poured and 0.9 cm wells were cut after solidification. After 1 h incubation, plates were flooded with 1% aqueous congo red and the plates were left to stand for 20 min. The plates were destained with 1 M sodium chloride solution and a clear zone against dark red background denoted cellulase activity [24,25].

Esterase activity: Plates containing (g%): tween 20, 1; phenol red, 0.01; agar, 18 were used after adjusting the pH to 6-7. The esterase activity will change the pink color to lemon yellow on account of the release of fatty acids [11,26].

Lipolytic activity: Agar plates containing 1 g% tween 80 and phenol red pH indicator were prepared. After incubation, positive lipase activity was noticed by changing the color to yellow [11,26].

Proteolytic activity: Plates with 1% skimmed milk and agar were utilized. After 24 h incubation, a clear zone was measured to reflect the extracellular protease activity [27].

Urease activity: Agar plates containing 1 g% urea and phenol red pH indicator were prepared. After incubation, the positive urease activity was noticed by changing the color of the medium [7].

A negative control, for every experiment, was done by adding the supernatant extract from unfermented media. The zone of activity was recorded after subtracting the –ve control zone, if any.

Results and discussion

Large amounts of waste residues, estimated over 950 million tons annually, are produced secondary to agricultural and complementary processes. These are materials that have no food or feed roles while posing an environmental and economic load. The random and uncontrolled disposal of waste, through dumping, landfilling and open burning, has created a major air pollution problem, eutrophication, greenhouse gas emissions and climate changes. Moreover, perturbations in the biological, chemical and physical soil nature also emerged [14,15]. In this regard, global efforts were designated to encourage circular economy and recycling material across different manufacturing processes, substituting non-renewable and virgin materials. This has been reinforced by the fact that using agro-byproducts as raw material for microbial enzymes production leads to overall industrial process cost reduction, serves the necessary microbial nutritional needs through the rich bioactive constituents level, and provides appropriate anchorage for the growing microbial cells [15,16]. Therefore, they represent a valuable candidate for cultivation of microorganisms, specially molds, by SSF.

Enzymes are competent biocatalysts that are gradually being employed in industry and widely used commercially replacing chemical processes due to their many advantages [1,5]. To boost their low operational cost quality, different waste materials with nutritional value could participate in producing the industrial enzymes.

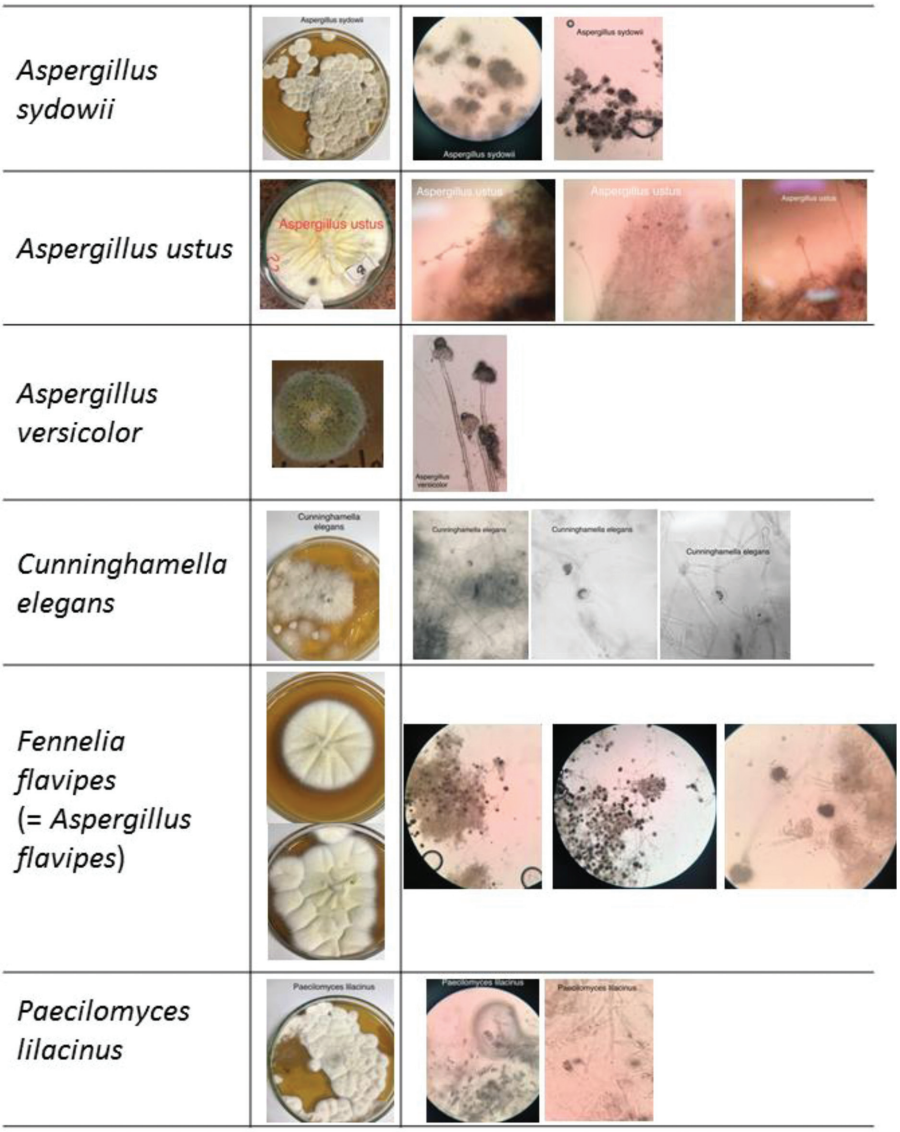
Therefore, this study was conducted to corroborate the capacity of incorporating waste materials in different industries depending on the relevant enzyme. Soil fungi were isolated due to the widespread use of their enzymes in industry, mounting up to almost 50% of the commercially applied ones, and their ability to degrade lignocellulosic material to simple substances that support their growth giving rise to high extracellular enzymes productivity [1,15].

Morphological determination of the isolated filamentous fungi

Six different fungal strains belonging to 4 genera were successfully cultured and morphologically identified from collected samples. The isolated strains belonged to the species: *Aspergillus sydowii*, *Aspergillus versicolor*, *Aspergillus ustus*, *Cunninghamella elegans*, *Fennelia flavipes* (= *Aspergillus flavipes*) and *Paecilomyces lilacinus* (Fig. 1).

A. versicolor, *A. sydowii*, *A. ustus* and *F. flavipes* (anamorph: *A. flavipes*) belong to class Eurotiomycetes, order Eurotiales, family Tricocomaceae. *A. versicolor* and *A. sydowii* belong to *Aspergillus versicolor* group whose species are differentiated according to their conidial heads [21,28]. *A. versicolor* and *A. sydowii* are capable of growing on various media including PDA on which the growth is fast with heavy colonies. On CZA media,

Figure 1



Terrestrial fungi isolated from the National Research Centre herbarium's soil; 4-days old colonies on potato dextrose agar (left) and mycelial growth under microscope (right).

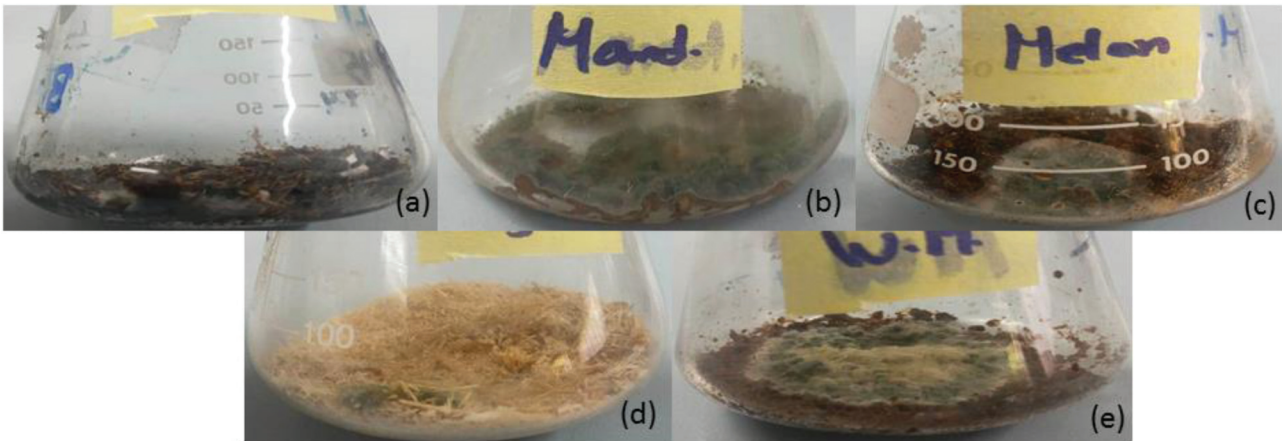
however, *A. sydowii* colonies attain 1.7–2.0 cm diameter, *A. versicolor* develops 1.5 cm diameter colonies, *A. ustus* colonies are dull brown to olive grey attaining 3.5–4.0 cm diameter, and *F. flavipes* possesses white to yellow shaded colonies of 3.5 cm diameter after 7 days incubation at 25°C [21].

Under microscope, *A. versicolor* and *A. sydowii* have conidial heads radiating with different colors, metulae, globose conidia and are echinulate. *A. versicolor* colonies are distinguished with different colors while those from *A. sydowii* are blue green [21,28]. *A. ustus* and *F. flavipes* show conidial heads radiating to loosely columnar form, present metulae and globose to subglobose conidia. While *A. ustus* presents smooth to roughened conidia, *F. flavipes* is characterized by smooth globose to subglobose conidia and smooth yellowish conidiophores [20].

P. lilacinus belongs to class Sordariomycetes, order Eurotiales, family Trichocomaceae. It grows on CZA giving colonies of 4.2 cm diameter after 7 days at 25°C. The margent is whitish and thin, conidial area is lilac to red-purple. Phialides consist of a cylindrical basal portion tapering to a long slender neck. Phialides are born singly on vegetative hyphae or in clusters on well-developed conidiophores [20,21,28].

C. elegans belongs to order Mucorales, family Cunninghamellaceae. Colonies completely fill malt

Figure 2



Growth of *Aspergillus versicolor* on agricultural waste materials via solid-state fermentation using: (a) banana peel, (b) mandarine peel, (c) melon peel, (d) sugarcane bagasse and (e) water melon peel.

extract, PDA or CZA plates rapidly after 3-5 days incubation at 25°C. *C. elegans* is globose, produce a single smooth spore that is ovoid or elliptical, verrucose or echinulate, hyaline to mostly heterothallic. Mycelium is white to dark grey reaching up to 3 cm height, nonseptate and often gets septated by aging, floccose and broad up to thick hyphae. Sporangiophores (conidiophores) are erect, branched verticillately or irregularly, they are usually swollen at point of attachment of lateral branches that terminate in globose, obovoid pyriform to clavate vesicle furnished with small smooth sporangioles (conidia) bearing denticles. On the other hand, zygospores are globose to somewhat flattened, dark brown, tuberculate, and with equal suspensors. Mature colonies are greyish white with terminal vesicles up to 40 µm diameter [20,21,28].

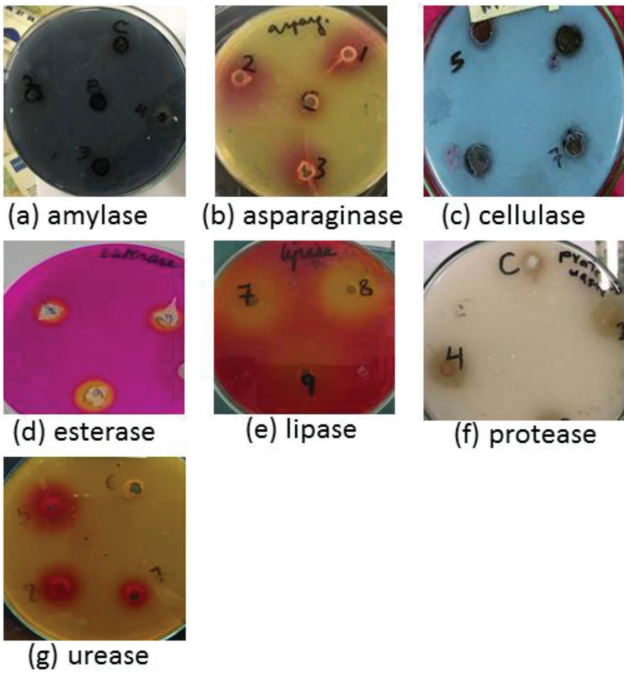
Enzymatic activity of the tested fungi

The six isolated fungi were cultivated via SSF on different waste materials (Fig. 2). The production of extracellular hydrolytic enzymes was noted upon cultivation on different agro-residues used as sole production medium, vis-à-vis standard medium (Figs 3 and 4). These enzymes were previously reported as both inducible and, to a lesser extent, constitutive. This investigation represented an attempt for the valorization of food wastes in biotechnological procedures that could be further extrapolated in other industries e.g. textiles, food, detergents, etc.

Amylolytic activity: The highest activity was recorded from *A. ustus* upon growing on minimal medium, using glucose as a single carbon source, which exhibited a lower activity when grown on melon peel. Likewise, *A. sydowii* showed amylolytic activity on minimal media, melon and banana peels. Banana peels also sustained equal amylase production from *A. versicolor* and *C. elegans*, the latter depicted a slightly lower activity after being cultivated on mandarine peel and sugarcane bagasse (Fig. 4a).

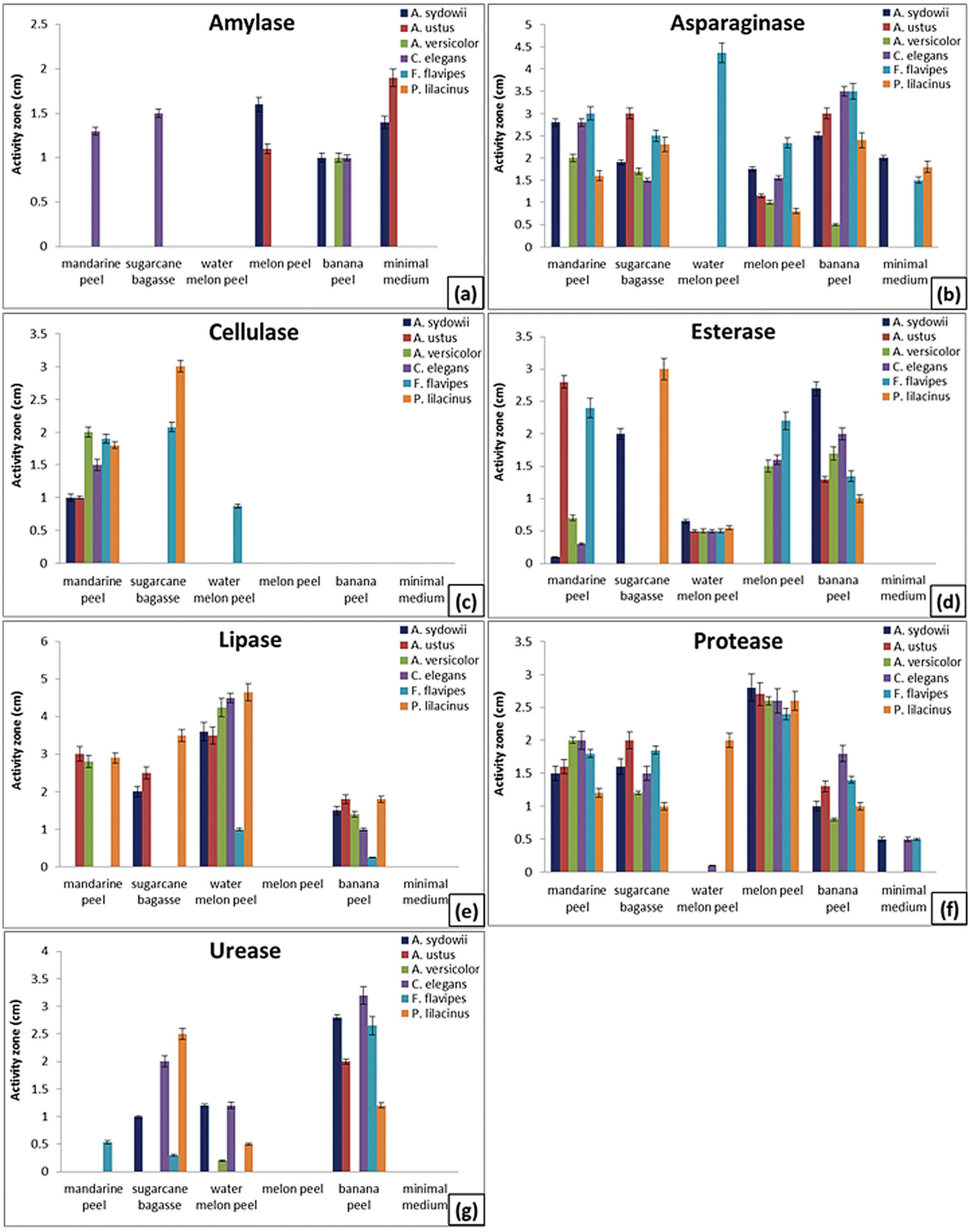
Asparaginase activity: The minimal medium could provide for the asparaginase production from

Figure 3



Agar-plate screening of hydrolytic enzymes: (a) amylase, +ve sample appear as clear zone; (b) asparaginase, +ve sample appear as red zone; (c) cellulase, +ve sample appear as clear zone; (d) esterase, +ve sample appear as yellow zone; (e) lipase, +ve sample appear as yellow zone; (f) protease, +ve sample appear as clear zone and (g) urease, +ve sample appear as red zone.

Figure 4



Hydrolytic enzymes' activity of the different fungal species isolated from local soil samples.

A. sydowii, *F. flavipes* and *P. lilacinus*. Variable asparaginase biosynthesis potential was also recorded by *A. sydowii* and *F. flavipes* when grown on all the tested wastes. In the case of *A. sydowii*, the highest activity was linked with the banana peels and the lowest with melon peel while *F. flavipes* exhibited the highest activity when grown on water melon peel and the lowest on melon peel, too (Fig. 4b).

Cellulase activity: Minimal medium, banana and melon peels were not able to support any cellulase activity from the tested fungi, under the test

conditions. Mandarine peel, on the other hand, helped the production of cellulase from all the tested fungi. *F. flavipes* and *P. lilacinus* were able to exert the highest recorded activity when grown on sugarcane bagasse (Fig. 4c).

Esterase activity: Except for very low activity by *P. lilacinus*, minimal medium did not support any esterase activity from the tested fungi, under the test conditions. Banana and water melon peels, however, sustained the activity from all the tested fungi, despite being very low in the second case (Fig. 4d).

Lipolytic activity: Like the aforementioned results for esterase enzyme, very low lipolysis was noted for *P. lilacinus* when grown on minimal medium. Else, no other activity was observed from it. Similarly, banana and water melon peels induced lipolytic activity from all the organisms yet, the latter was roughly three times the activity of the former. Conversely, melon peel did not nurture any lipase production (Fig. 4e).

Proteolytic activity: *C. elegans* could digest proteins when grown on all the tested media, despite being extremely poor upon water melon peel cultivation. All the tested agro-residues, except for water melon peel, showed proteolysis from all the tested fungi while the highest results were obtained from melon peel (Fig. 3f).

Urease activity: Minimal medium, mandarine and melon peels did not support urease activity, under the test conditions, while banana peel showed the best results for five out of six tested organisms. *A. sydowii*, *C. elegans* and *P. lilacinus* showed urease activity when cultured on sugarcane bagasse and water melon peel (Fig. 4g).

Fungi are an integral component of grassland's ecosystem on account of their active engagement in the biotic and abiotic interactions e.g.: nutrients cycles, biochemical activities, etc. Hence, they directly play a major role in plants' production and resilience against drought and pathogens. The fungal community is, in turn, influenced by the edaphic conditions e.g.: moisture level, temperature, plant diversity, etc. It is postulated that the richer the diversity of soil fungi, the more stable and productive the ecosystem. One plausible explanation thereof is their participation in yielding ample amounts of nutrients for plant growth [29–31]. In this study, the prevalence of the saprophytic members of phylum Ascomycota and order Eurotiales was clear, as previously documented in reports describing fungal diversity in shallow soil layers, due to their tolerance to harsh physicochemical conditions and their high competence to decompose organic and xenobiotic matter [30–32]. Four out of six different isolated pure strains belonged to *Aspergilli* species, agreeing with the previous conviction that they are the most prevalent genus that can be isolated from soil and plant remainings in different surveyed locations and environmental habitats [33,34].

Since ancient times, agriculture has been considered a fundamental activity and cornerstone to the Egyptian economy. Therefore, exploiting the huge amounts of fruit and vegetable wastes that are produced and disposed constitutes a crucial environmental and economic necessity. Besides, local sourcing is greatly beneficial with regards to higher revenue, better procurement control, ecosystem impact and administrative framework. Hence, the herein investigated agro-residues arise from locally-grown and abundant plants. In general, agricultural activities result in massive amounts of waste, both after field or industrial processing. The wastes that are rich in nutritive components, e.g.: complex polysaccharides, gums, vitamins, starch, cellulose, hemicellulose, lignin, minerals, carbon and nitrogen, are thus preferably utilized instead of being totally lost e.g.: animal feed, biogas, biofuel, biosurfactants, single cell proteins, antibiotics production, etc. Moreover, in this report, no waste pretreatment or biorefinery was performed to test for the possibility of even further cost reduction, limited need for complicated preparatory handling and less labor engagement.

Banana peel is rich in carbohydrates and calcium where carbohydrates represent 60% of its weight, most of which is starch while calcium represents 30%. Since most α-amylases are known to be calcium-dependent [16], banana peel is a potentially good candidate for the enzyme's production. It has indeed been previously used to produce α-amylase from the saprobes *Aspergillus niger* and *Rhizopus oryzae* [16,35,36]. Interestingly, amylase activity was more superior when obtained via SSF than SmF due to the associated production of non-specific proteins [16]. L-asparaginase was also detected from *Fusarium oxysporum* by SSF, with higher titers compared with SmF, using several waste substrates, including banana peel [22]. Laccase, pectinase and xylanase are other enzymes that have been produced using banana substrate, as well [18,37]. On the other hand, some fungal isolates e.g.: *Fusarium culmorum* and *Xylaria curta* were not able to grow on the same substrate on account of its antibacterial properties [8,27]. Citrus fruits belong to the family Rutaceae and are considered among the most prevalently cultivated fruit families

and the peels form almost 50% of the fruit mass. They are known to be rich in phenolic flavonoids and fibers. Like the whole peel (epicarp and mesocarp) used in this study, mandarine and orange peels were used as single carbon sources to produce the hydrolyzing enzymes; cellulase, laccase and xylanase, from various *Fomes* spp., *Ganoderma* spp. and *Pleurotus* spp. basidiomycetes [38]. Lemon and orange peels supported the production of L-asparaginase from *Fusarium oxysporum* in the near absence of free water by SSF [22]. Also, the highly demanded α-amylase was produced from *Aspergillus flavus* grown on mandarine peel using SmF [6]. Similarly, sugarcane bagasse represents 30% of the processed stem [39]. When used as a sole carbon source for several filamentous fungi growth, different levels of amylase, glucanase and pectinase from *Aspergillus japonicus*, cellulase and β-glucosidase from *Aspergillus japonicus* and *Fusarium oxysporum* were measured [39]. *Aspergillus flavus* also grew on sugarcane bagasse and tested positive for cellulase and xylanase activity [19].

Nitrogen is a macronutrient for all living organisms and is essentially applied in fertilizers in crop fields e.g.: composted dead material, urea-based synthetic fertilizers, animal manure, etc. On account of the high stability of urea, its degradation is minimal in the absence of the nickel-dependent urease enzyme [40]. Thereby, the presence of urease activity could be attributed to the fungi's response to heavy metal contamination, residual fertilizers or arginine hydrolysis during the urea cycle e.g.: *Aspergillus niger* producing hexameric urease when cultivated by SSF on wheat straw [13,40].

Several studies are available on screening of enzymes from microorganisms inhabiting various environmental sources for industrial applications. However, some limitations may face those studies, such as considering the suitable procedure and the looked-for characteristics for instance rapid growth rate, biosafety worries as well as cheap nutrient necessities. Besides, down streaming of enzymes and their purification may be of high cost.

Thus, it is recommended to better understand and design treatment process such as immobilization which may improve the reusability and stability of industrial enzymes. Also, recombinant enzymes overexpression when combined with the appropriate expression vector can boost output.

Conclusion

In comparison to plants and animals, microbial metabolites are a rich mine that can serve different industries on account of their high productivity, easy up-scalability and functional optimization via genetic modification and protein engineering. Therefore, the endeavors of examining new niches to identify products with unique characteristics are perpetual. Furthermore, the screening in solid media is an efficient and practical tool that aids the quick selection and comparison of enzymes' production by various isolates. SSF has afforded efficient microbial growth and simple product recovery. Additionally, agriculture and forestry's residual wastes are valuable sources of macro- and micronutrients for microbial cultivation and their use is an eco-friendly, clean and inexpensive method to generate commercially valuable biomass and products. The recycling of wastes into high-value end products is a meaningful step towards sustainability and environment protection to curb the repercussions of climatic changes, energy crisis, environmental degradation and population increase.

Abbreviations

Czapek's agar, CZA; Potato dextrose agar, PDA; solid-state fermentation, SSF; submerged fermentation, SmF.

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Ethics committee approval

This work has been granted the final approval of the Medical Research Ethics Committee (MREC) after satisfying the guidelines and recommendations, under number 11444052023.

Authors Contributions

Conceptualization, H.Y., R.A.; Methodology, A.S., H.Y., R.A.; Investigation, A.S., H.Y., R.A., W.E.; Data curation, H.Y.; Writing- original draft preparation, H.Y., A.S.; Visualization, H.Y. All authors have read and agreed to the published version of the manuscript.

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Nil.

Conflicts of interest

The authors declare that they have no conflict of interest.

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