Current Research in Microbial Sciences 7 (2024) 100254

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Recovery of filter paperase from mouldy rice husk in solid state fermentation by Aspergillus protuberus

P.Suresh Yadav [a], B.V.Siva Prasad [a], M.Subhosh Chandra [a,*], M. Srinivasulu [b], Naga Raju Maddela [c], Ram Prasad [d,**]

[a] Department of Microbiology, Yogi Vemana University, Kadapa, Andhra Pradesh, India
[b] Department of Biotechnology, Yogi Vemana University, Kadapa, Andhra Pradesh, India
[c] Departamento de Ciencias Biológicas, Facultad de Ciencias de la Salud, Universidad Técnica de Manabí, Portoviejo, Manabí, Ecuador
[d] Department of Botany, Mahatma Gandhi Central University, Motihari 845401, Bihar, India

ARTICLE INFO

Keywords:
Aspergillus protuberus
FPase
Extraction
Solid state fermentation
Rice husk

ABSTRACT

Recovery of enzymes such as FPase (filter paperase) or exoglucanase from fermented substrate is a sustainable approach in enzyme production; however, there is a scarcity of optimization studies in this field. The present study was aimed to standardize number of parameters (selection of solvent, solvent volume, soaking time, leaching conditions and number of washes) to extract maximum amount of FPase from fermented rice husk by *Aspergillus protuberus*. Novel *Aspergillus protuberus* was first report from our lab on cellulases production in solid state fermentation (SSF). Among the tested solvents, citrate phosphate buffer (0.02 M, pH 5.0) proved best solvent for maximum recovery of FPase. Consequent experimental parameters were further optimized with citrate phosphate buffer. Two washes with citrate phosphate buffer each by shaking (60 min) in a ratio of 1 g of rice husk: 5 ml of citrate phosphate buffer together attained higher recovery efficiency (88 %) of FPase from the fermented rice husk.

Introduction

Fossil gas depletion and the escalation of global transportation fuels petition has diagnosed priority efforts toward finding opportunity renewable biofuels and 2nd generation technology (2 G) biofuels has lately been an international research consciousness (Brar et al., 2019). But, maximum 2 G biofuel technology is nonetheless on the door, because of their excessive fee of saccharification enzyme, particularly cellulase (which breaks down cellulose into glucose) represents approximately 30 % of the whole manufacturing cost (Bernardi et al., 2023). Cellulose accounts 30–40 % of the structural framework of lignocellulosic biomass, at the side of 20–30 % hemicelluloses, and 20–30 % lignin (Obeng et al., 2020). However, maximum fermentative microbes are not able to metabolize it immediately and monomeric sugars are alternative (Pandey and Negi, 2020). Consequently, the breakdown of cellulose to glucose entails 3 cellulase components, along with endo-glucanase (targets the amorphous region and randomly cuts inner bonds to show new ends within the cellulose polymer), exoglucanase (breaks uncovered ends to form di or tetra-saccharides), and β-glucosidase (cuts disaccharides into glucose) (Pandey and Negi, 2015). Consequently, a fee-powerful cellulases with excessive hydrolysis capability is the important thing to unlocking cellulose for use in biofuels, fermentation, cultivation, paper, fabric, and feed industries (Ranjan et al., 2023). Different solid substrates were used for production of cellulases (Yadav et al., 2016; Shruthi et al., 2018). Douglas fir forest waste was used as a substrate for production of higher titer and higher yield of bioethanol by sulfite chemistry without separation of solid–liquor and detoxification (Zhu et al., 2015).

Every year, India generates a big quantity of stable waste wherein AW stays on the top with approximately 350–990 Mt/12 months. Next to China, India is the second largest country to produce agrowaste in global, generating greater than one hundred thirty million tonnes of paddy straw, half of which is used as animal feed and alternative 1/2 of which is disposed (Singh and Sidhu, 2014). Further, the practice of burning rice waste inside the northwestern area produce great air pollution and increases community health worries (Shyamsundar et al.,

* Corresponding author at: Department of Microbiology, Yogi Vemana University, Kadapa, 516005, Andhra Pradesh, India.
** Corresponding author at: Department of Botany, Mahatma Gandhi Central University, Motihari 845401, Bihar, India.
E-mail addresses: subhash_muni@yvu.edu.in (M.Subhosh Chandra), ramprasad@mgcub.ac.in (R. Prasad).

https://doi.org/10.1016/j.crmicr.2024.100254

Available online 21 June 2024
2666-5174/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2019). Unsuitable discarding of crop residues ends in formation of greenhouse gases (GHG) which includes CO_2, nitrous oxide N_2O and CH_4, which pose a danger to humans and herbal environment (Kaab et al., 2019). The methods currently used related to agro-wastes still hold some disadvantages. Environmental pollution occurs when unplanned landfilling and burning of wastes for energy production (Sadh et al., 2018), their utilization as animal feed cause to low digestibility and antinutritional factors (Tripathi and Mishra, 2007). Biotechnology is a powerful tool to convert agricultural wastes to value-added products. Agrowastes are usually considered the best solid supports for SSF (Pandey et al., 1999). A number of agroresidues are used as solid supports for cellulolytic enzymes production such as sesamum husk, sugarcane bagasse, tea residue, castor husk, saw dust, groundnut fodder and rice husk (Shruthi et al., 2018), groundnut fodder, rice bran, wheat bran and sawdust (Chandra et al., 2007; 2022; Chandra and Reddy, 2013), rice husk (Yadav et al., 2024)

Presently, first-era feedstocks (molasses and starch) are the primary participants to worldwide bioethanol production; but, because of the growing demand for bioethanol and the plentiful availability of cheap lignocellulosic biomass (LCB), attention is shifting to the improvement of cellulosic ethanol production technology (Brar et al., 2019). Fungus and bacterium were identified to produce cellulase on cellulosic biomass, of which fungi accounted for 80 % of SSF (Pandya and Gupte, 2012). SSF mimics herbal environment for production of cellulase in higher amounts by fungi (Santos et al., 2017). It ought to be noted that the restoration method of cellulose is simpler than membrane-bound intracellular cellulosomes (ICs), where the subsequent step of cellular wall breakdown is vital. In addition, decrease water and power requirements with the least waste technology in SSF majority of the industries employed SSF than submerged fermentation (SmF) for cellulase production (Ezeilo et al., 2019). But, designing scaling methods in SmF below controlled factors is relatively less difficult than SSF (Kumar et al., 2021). Most cellulase-generating SSF technology has specifically centered on upstream approaches (Bagewadi and Ninnekar, 2015). However, higher titre of cellulose in SSF is too a characteristic of effective leaching from SSF and needs to be attended (Dey et al., 2021). As a consequence, for a high yield of cellulase in SSF, a standardized leaching process is required to reap most cellulase from mouldy substrate in lively and intense form.

SSF is rapidly gaining interest for financial enzyme manufacturing due to its price-powerful generation, low capital investment, and clean managing technology (Chandra et al., 2010). Enzyme leaching from the mold substrate is important in SSF (Pragya, Krishna et al., 2023) and is of key importance. Therefore, the prevailing examine targeted at the standardization of extraction parameters for maximum leaching of exoglucanase, which is one of the key components of cellulolytic enzymes produced with the aid of *Aspergillus protuberus* in SSF.

Materials and methods

Fungal strain

Aspergillus is an expansive genus consisting of approximately 250 species; they are categorized into seven sub-genera that are in turn subdivided into several sections. Our laboratory, we isolated *Aspergillus* strain from decomposing forest soil samples. Identification of strain was based on morphological, microscopic and macroscopic characteristics. Lactophenol cotton blue staining was done to observe the strain under a microscope. Colony morphology, i.e., characteristics of mycelia, conidia, and conidiophores were observed in light microscopy as well as Scanning Electron Microscope (SEM). The species produce colonies, composed of white or yellow flat that is covered by dark asexually produced fungal spores and it was identified first time as *Aspergillus protuberus* based on morphological and molecular characteristics such as 28S rDNA sequencing and deposited in GenBank under the accession number **KX427028** (Yadav et al., 2017). Pure culture was maintained on Czapek Dox medium at 30±2 °C. The culture was stored at 4 °C. Inoculum was arranged from 7 days grown slants with sterile distilled water consists of Tween-20 (0.2 % v/v). Spore count was calculated by hemocytometer and adjusted to 2×10^5 spores/ml by adjustment of optical density.

Lignocellulosic substrate

Rice husk was procured from sawmill in Kadapa. The husk was air dried and sieved through 2 mm screen to get consistent size of particle.

Solid state fermentation

Ten grams of rice husk was placed into 250 ml conical flasks and sterilized at 121 °C for 15 min (Chandra and Reddy, 2013; Shruthi et al., 2018). The sterile rice husk was retained with 40 % (w/v) of moisture. The modified Czapek Dox liquid medium contains components (g/L) K_2HPO_4 1.0, $MgSO_4{\cdot}7H_2O$ 0.5, KCl 0.5, $FeSO_4{\cdot}7H_2O$ 0.01, glucose 10, cellulose 5.0, $(NH_4)_2SO_4$ 20, distilled water 1000 ml, pH 5.0. Sterile substrate in flasks were inoculated with spores of 2×10^5 spores/flask and kept at ambient temperature i.e., 30±2 °C up to 5 days.

Leaching technique

Fermented husk in the conical flask was recovered with chosen solvent at 30 °C. The crude extract was separated through cotton cloth and obtained filtrate was centrifuged at 10,000 rpm at 4 °C and supernatant was employed for enzyme assay.

Filter paper assay

The filter paperase activity extracted in leachate was estimated by Mandels and Weber (1969). Fifty milligram Whatman filter paper strip (1 × 6 cm) was immersed into 1 ml of 0.05 M sodium citrate buffer (pH 4.8) at 50 °C in a water bath. Crude enzyme was mixed with above solution and incubated for 60 min at 50 °C. After incubation, 3, 5-dinitrosalicylic acid (DNS) was added. All samples were mixed thoroughly and boiled for 5 min in a boiling water bath. After cooling, color formation in tubes was read at 540 nm in UV spectrophotometer. One unit of filter paper unit (FPU) was expressed as the amount of enzyme liberating 1 μmole of reducing sugar from filter paper per min. Activity of cellulase was expressed in filter paper units.

Statistical analysis

The data represented is the averages of reproduces. Duncan's Multiple Range (DMR) test was employed for the complete data done by Megharaj (1999). The data was analyzed by Repeated ANOVA test ($P < 0.05$) to know the significant effects of different solvents, volume of the citrate-phosphate buffer, and number of washes on FPase activity. One sample *t*-test analysis (α 0.05) was done to know the significance of stationary and static conditions on the FPase activity. GraphPad Prism 9.0.0. program was used for the statistical analysis of the data.

Results

Selection of extraction solvent

In SSF, a suitable solvent is required to extract higher amount of enzyme with minimal soaking time (Adhyaru et al., 2015). The recovery efficacy of solvent is mainly depends on nature of fungal mycelium and its interaction with the enzyme (Fernandez - Lahore et al., 1998).

SSF has many advantages than submerged fermentation, such as high volumetric productivity, less waste discharge, and low catabolic repression which makes it a gifted approach in near prospect. Hence it is strongly recommended technology for production of cost-effective and

high concentrated bio-catalysts. Amount of extracting of these concentrated biocatalysts from mouldy substrate are influenced by various extraction parameters. Therefore, some extraction parameters, i.e., solvent efficacy, ratio of solid to solvent, time for soaking, physical state of leaching and washings were optimized for effective leaching of FPase from mouldy rice husk using *A. protuberus*.

High titers of FPase (2.67 FPU/g of rice husk) from the 1-day fermented husk with citrate-phosphate buffer (Fig. 1, **ST. 1, SD. 1**). Followed by 2.61 FPU/g of rice husk in leachates of citrate buffer, 2.47 FPU/g rice husk in leachates of 5 % methanol and 2.45 FPU/g of rice husk in leachates of acetate buffer. Among the solvents used in the present study, distilled water made poor recovery of FPase with 1.74 FPU/g of rice husk from 1-day mouldy husk.

Solid to solvent ratio

SSF is a static fermentation of fungus growth on moist solid substrates where value added products are produced in a concentrated manner, because of less solvent. Therefore, sufficient volume of solvent is required to extract maximum of the product. Therefore, fermented husk to solvent ratio is other significant factor to at most leaching of enzyme in SSF. The volume of solvent i.e. citrate phosphate buffer from 20 to 50 ml with 10 ml span, extraction was performed directly after the addition of solvent.

Fifty milliliter of citrate-phosphate buffer achieved recovery of FPase in maximal titer of 2.45 FPU/g of rice husk (Fig. 2, **ST. 2, SD. 2)**. The corresponding figures of FPase extraction from fermented husk with 40, 30 and 20 ml of citrate-phosphate buffer were 2.44, 2.09 and 1.98 FPU/g of rice husk. Extraction of FPase from fermented husk was depending on volume of the solvent used. Five-day old fermented husk gave higher yields of FPase.

Time for soaking

Once optimized the solvent type and its volume at optimal level, time for soaking was standardized for maximum recovery of FPase from mouldy rice husk. Contact time of mouldy substrate and leaching solvent are another crucial factor, which needs to be standardized. The time intervals used in the present study was from 30 −120 min with the span of 30 min (Fig. 3, **ST. 3, SD. 3**) for efficient recovery of enzyme where all other conditions were same as described in SSF.

Soaking of 1-day old fermented husk with 60 min gave higher yields of FPase with 5.53 FPU/g of rice husk as against 5.43 FPU/g of rice husk by 30 min soaking of fermented husk of the same age (Fig. 3). Soaking of 3-day incubated fermented husk with 120 min yielded less FPase activity with 3.80 FPU/g of rice husk on 3rdday of incubation. Maximum yields of FPase occurred in 1-day fermented husk.

Physical state of leaching

Shaking conditions were effective in extraction of FPase from fermented husk than stationary conditions (Fig. 4, **ST. 4, SD. 4 and SD. 5**). Application of shaking conditions during soaking of 1-day fermented rice husk improved FPase recovery with 2.32 FPU/g of rice husk as against 1.70 FPU/g of rice husk under stationary conditions. Peak production of FPase in 1-day fermented husk in this experiment.

Number of washes

In previous experiments, leaching of enzyme from mouldy husk was employed with a single washing of the solvent, citrate phosphate buffer. In order to test whether a single wash could completely extract the enzyme, the fermented husk recovered after the first washing was again subjected to repeated washing with fresh lots of solvent. Washings were collected separately and assessed for the chosen parameters.

Higher FPase activity with 3.24 FPU/g of rice husk was recovered in the first wash from 1-day old fermented husk (Fig. 5, **ST. 5, SD. 6**). The second wash extracted FPase to the extent of 1.28 FPU/g husk from once extracted fermented husk. FPase leached into third wash of fermented husk to the extent of 0.68 FPU/g of rice husk. The corresponding figures of extraction by the first, the second and the third wash from 1-day old fermented husk were 3.24, 1.28 and 0.62 FPU/g of rice husk. Total yields of FPase in 1-day old fermented husk were 5.2 FPU/g of rice husk touching peak.

Discussion

Rice is common food for almost half of the global population (Slayton and Timmer, 2008). After wheat and corn, rice is the third most significant grain crop in the globe in total production. During the milling the harsh coat of the rice grain is removed as a byproduct. Among all the agrowastes, the availability of rice husk is easy throughout the globe.

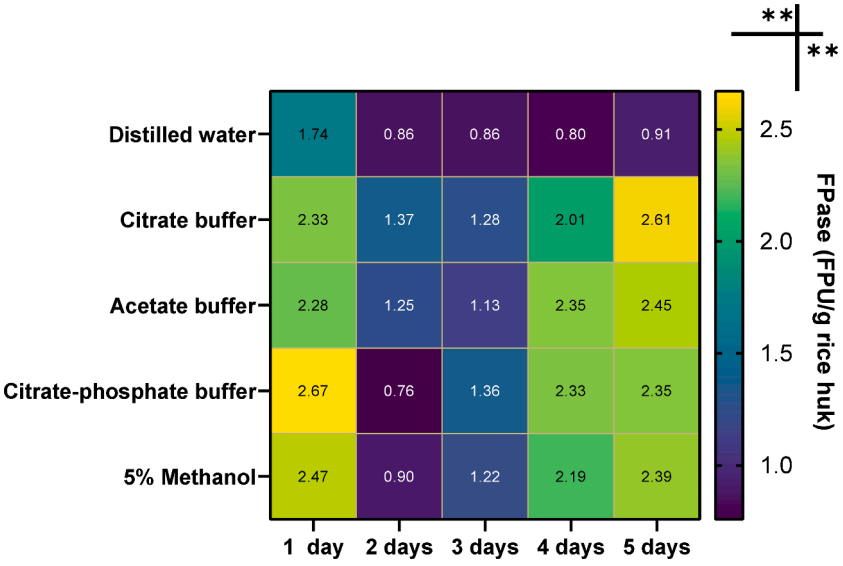

Fig. 1. Recovery of FPase from fermented rice husk by different solvents. There was a significant[#] effect of time ($P = 0.0032$) and solvent ($P = 0.0016$) on the FPase activity. [#]Repeated measure ANOVA analysis ($P < 0.05$). Asterisk with horizontal line indicates row data (i.e. solvents) and asterisk with vertical line indicates column data (i.e. incubation in days).

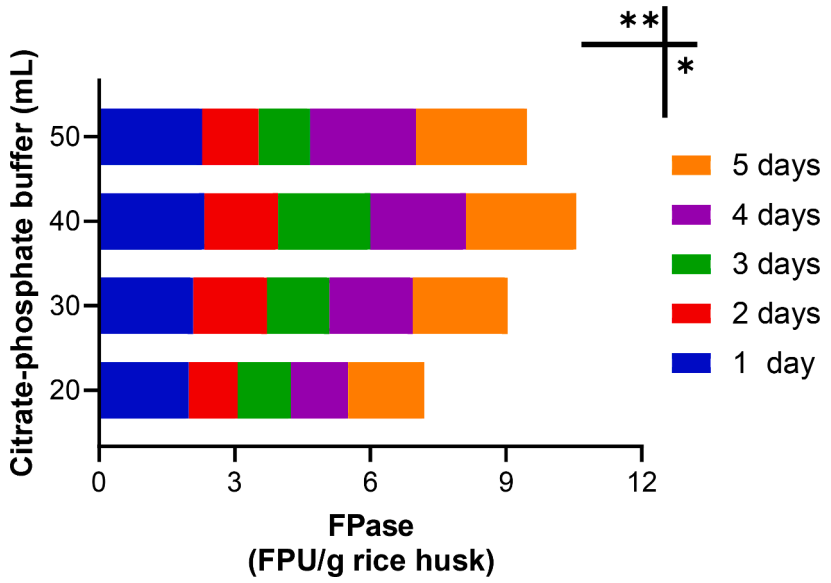

Fig. 2. Recovery of FPase from fermented rice husk with different volumes of acetate buffer. Volume of the buffer ($P = 0.0069$) and incubation days ($P = 0.0199$) have significant[#] effect on the FPase activity. [#]Repeated measure ANOVA analysis ($P < 0.05$). Asterisk with horizontal line indicates row data (i.e. Citrate-phosphate buffer volume) and asterisk with vertical line indicates column data (i.e. incubation in days).

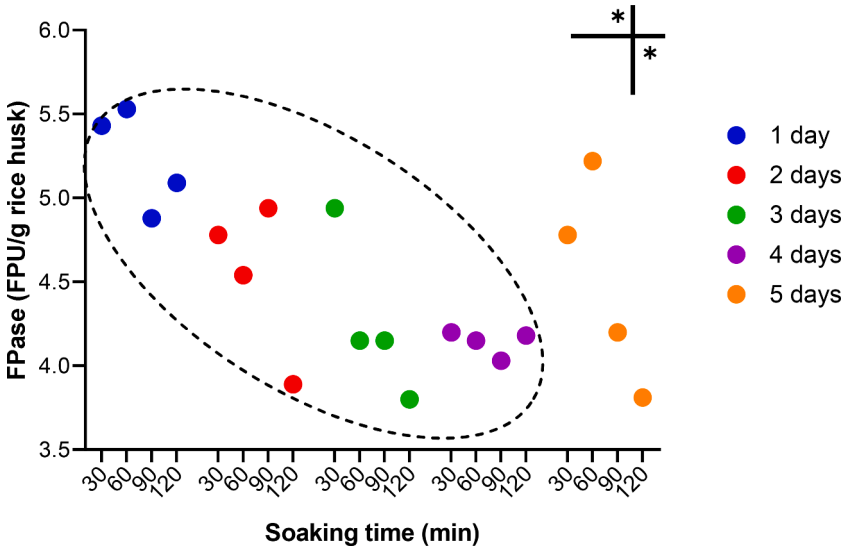

Fig. 3. Effect of soaking time on recovery of FPase from fermented rice husk. Incubation ($P = 0.0205$) and soaking time ($P = 0.0395$) have significant[#] impact on the FPase activity. [#]Repeated measure ANOVA analysis ($P < 0.05$). Asterisk with horizontal line indicates row data (i.e. Soaking time in min) and asterisk with vertical line indicates column data (i.e. incubation in days).

Worldwide, almost 600 million tons of rice produced every year.

In annual production of rice paddy husk (120 million tons), 20 % of the rice paddy is husk (Giddel and Jivan, 2007). Approximately half of the rice husk produced through rice mills are generally burned for the production of steam to run milling equipment. In addition, several reports shown that rice husk contains high amount of cellulose and hemicelluloses. Therefore, husk is also used to produce several value added products. In rice producing countries, rice husk used as a biomass/solid support is an attractive way for production of bioethanol. Rice husk consists 75–90 % organic matter i.e., cellulose, hemicellulose, lignin etc., and silica, alkalis, and trace elements (Wallheimer and Brian, 2010). It contains higher ash content than other substrates in the range of 10–20 %. In ash it consists about 87–97 % silica, highly porous and light weight, with higher external surface area. Presence of higher amount of silica makes it a valuable material for use in production of value added products (Sarangi et al., 2009). Rice husk also contains K_2O, Al_2O_3, CaO, MgO, Na_2O, Fe_2O_3 are available in less than 1 %. Rice husk contains 96–160 kg/m^3, oxygen 31–37 %, nitrogen 0.23– 0.32 %, sulphur 0.04–0.08 %. Because of these characteristics which make them suitable for production of bioethanol (Sudiyani and Muryanto, 2012). Campos et al. (2024) screened four different aqueous solutions of sodium chloride (0.9% w/v), calcium hydroxide (3.0 mM), Tween-80 (0.01 % v/v) and glycerol (10 % v/v) to select best solution to extract maximum amount of CMCase and FPase. Among the tested solutions, NaCl solution recovered higher CMCase and FPase. Darabzadeh et al. (2019) also used distilled water to recover cellulase from fermented substrate. Maximum cellulase extraction was achieved with distilled water from different solid substrates by Marin et al. (2019).

An ultimate solvent recovers enzyme particularly and totally at optimum temperature by minimum contact time (Chandra et al., 2008).

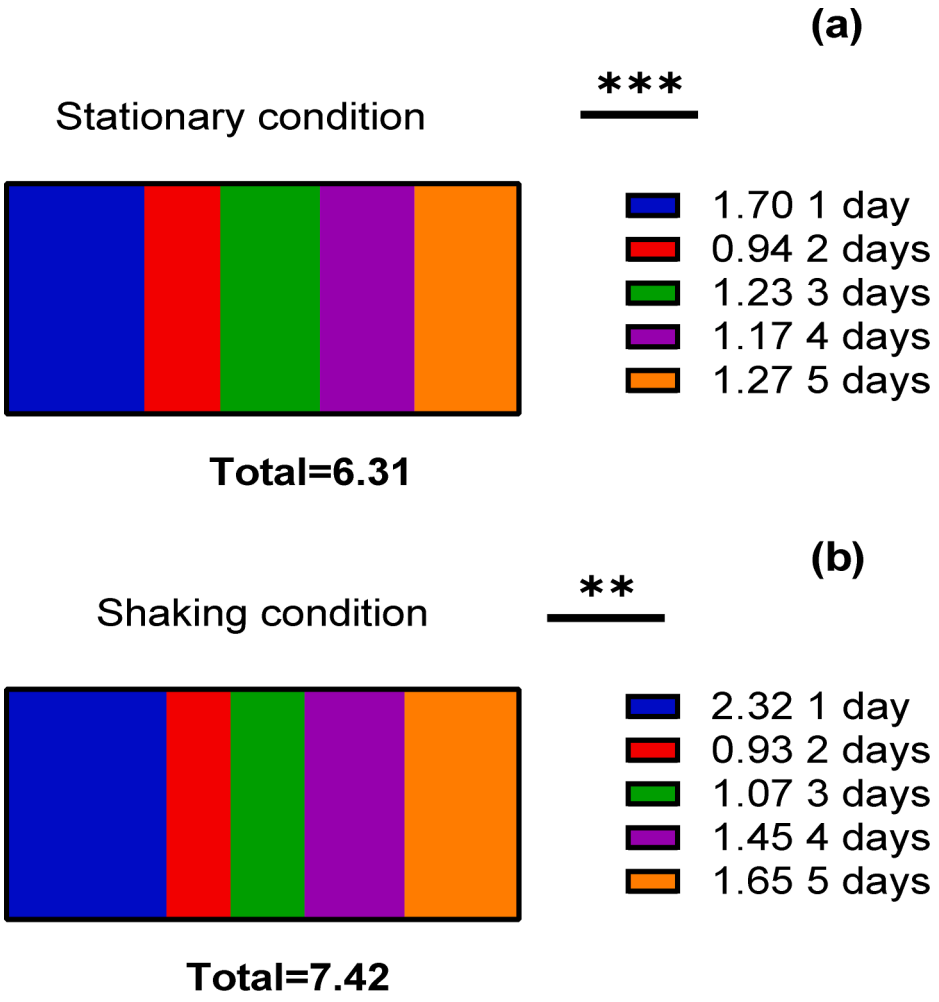

Fig. 4. Effect of different leaching conditions on recovery of FPase from fermented bran by acetate buffer. FPase activity was significantly[#] different between the incubation days in the stationary ($P = 0.0005$, R^2 0.9631) and shaking ($P = 0038$, $R^2 = 0.9013$) conditions. [#]One sample t-test analysis (α 0.05).

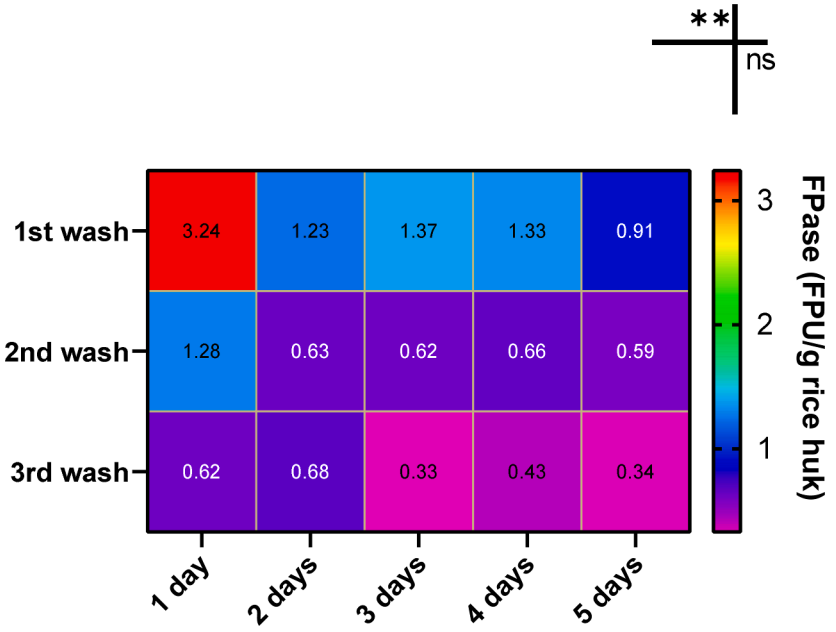

Fig. 5. Effect of number of washes on recovery of FPase from fermented bran by *A. protuberus* in SSF. FPase activity was significantly[#] changed between the washes ($P = 0.0096$), however, FPase activity was not significant ($P = 0.2311$) between the incubation days. [#]Repeated measure ANOVA analysis ($P < 0.05$). Asterisk with horizontal line indicates row data (i.e. number of washes) and 'ns' (not significant) with vertical line indicates column data (i.e. incubation in days).

Furthermore, recovery efficacy of a solvent is another significant attribute for complete leaching of enzyme from fermented substrate in SSF. Therefore, selection of an ideal solvent is essential for greater extraction of enzyme from SSF. Different solvent systems i.e. 0.2 M, pH 5.0 citrate buffer, acetate buffer, citrate phosphate buffer and 5 % methanol (v/v) were screened separately. Each wash of solvent by 50 ml of volume was used for recovery in experiment.

Diverse solvent systems were evaluated for leaching efficacy of various enzymes from mouldy substrates in various studies. Distilled water for FPase recovery from mouldy bran by *A. niger* in SSF (Chandra et al., 2008), 50 mM citrate buffer (pH 4.8) for leaching of CMCases, xylanase, and β-glucosidase from the spent mushroom compost of *P. sajorcaju* (Bisaria et al., 1990; Singh et al., 2003), glycerol 2.5 % (v/v) used for maximum leaching of α-amylase on wheat bran using *Bacillus circulans*GRS313in SSF (Palit and Banerjee, 2001). Fernandez Lahore (1998) were observed that hydrophobic or hydrophilic character of fungal growth, ionic bonds, hydrogen bonds and Vander Waal forces decides efficacy of solvent for enzyme leaching. The use of acetate buffer (0.2 M, pH 4.8) for extraction of endo-glucanase from mouldy wheat bran using *Aspergillus niger* in SSF was reported by Pirota et. al. (2013). Pandey and Negi (2020) evaluated the different solvent systems for the extraction of cellulase enzyme form the fermented wheat bran by a local isolate of *Rhizopus oryzae* SN5 can extract 18.9 % enhanced extraction of cellulase enzyme from fermented substrate when compared with other buffers used in the present study.

Solvent to substrate ratio of 10–11 ml/g was recorded as optimal ratio for higher extraction of xylanase by *A. niger* DFR5 by Pal and Khanum (2010). Hence the use of less substrate to solvent ratio is necessary to retain product in concentrate form to attain higher leaching efficiency. Castilho (2000) reported the 30 min soaking time is optimal for effective leaching of protease extraction by SSF. Soaking of fermented substrate in water for 1 h was used for leaching of cellulolytic enzymes (Fadel, 2000), but 150 min soaking for amylase recovery was noticed by Palit and Banerjee (2001). Pandey and Negi (2020) tried different solid to solvent ratio (1:6; 1:8; 1:10, 1:12 and 1:14) for find out suitable solid: solvent ratio and found that the 1:10 is the best combination for maximum extraction of enzyme from the fermented wheat bran by a local isolate of *Rhizopus oryzae* SN5.

Agitation effect is quite acceptable due to agitation of mouldy bran gets dispersed evenly in constant phase of solvent, minimizing polarization (Tunga et al., 1999). Dharmesh 2015 also extracted maximum recovery of xylanase enzyme from fermented sorghum straw by *A. tubingensis* FDHN1 strain at 150 rpm. In shaking conditions loss of enzyme activity caused by prolonged agitation was reported by Ghildyal (1991). Pal and Khanum (2010) observed the maximum extraction of xylanase enzyme at highest shaking (200 rpm). Similar results were also observed for xylanase recovery at high agitation of 200 rpm by Azin (2007). Complete recovery of β-glucosidase from mouldy husk enhances process efficiency. In the present study, first two washes collectively could account for 92 % of total extraction of enzyme. Similar results were observed by Palit and Banerjee (2001) on leaching of amylase. Pandey et al. (2020) tried various soaking times (30, 60, 90,120,150 min) for selection of optimum incubation time and found that the 90 min is the most excellent incubation time for maximum extraction of cellulase enzyme from the fermented wheat bran by a local isolate of *Rhizopus oryzae* SN5.

Conclusions

To the best of our knowledge, this is the first report on leaching of FPase from rice husk in SSF by *A. protuberus.* Recovery of product from complex fermented matrices place a significant role in downstream processing. With these standardized parameters in this study, we have recovered almost 90 % of FPase from the fermented rice husk in SSF by *A. protuberus*. Agrowastes utilization as animal feed leads to less digestion and they may have antinutritional elements. By overcome this with the aid of cellulases which will increase the digestion and degrade antinutritional elements. In this study untreated solid substrates were used. Utilization of pretreated solid substrates certainly increases the yields of FPase by microbes in SSF which needs to be further explored. This attempt certainly opens up the doors in downstream processing for the young futuristic research minds to precede research.

Ethical approval

This article does not contain any studies with human participants or animals.

Consent to participate

All authors agree mutually with the participation and publication of this work and declare that this is original research.

Consent to publish

All authors agree mutually to publication of this work.

CRediT authorship contribution statement

P.Suresh Yadav: Writing – original draft, Methodology. **B.V.Siva Prasad:** Writing – original draft, Methodology. **M.Subhosh Chandra:** Conceptualization, Supervision, Writing – review & editing. **M. Srinivasulu:** Data curation, Writing – original draft, Formal analysis. **Naga Raju Maddela:** Data curation, Writing – original draft, Formal analysis. **Ram Prasad:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare there is no conflict of interests.

Data availability

Data will be made available on request.

Funding

NA.

References

Adhyaru, D.N., Bhatt, N.S., Modi, H.A., 2015. Optimization of upstream and downstream process parameters for cellulase-poor-thermo-solvent-stable xylanase production and extraction by *Aspergillus tubingensis* FDHN1. Bioresour. Bioprocess. 2 (3) https://doi.org/10.1186/s40643-014-0029-1.

Azin, M., Moravej, R., Zareh, D., 2007. Production of xylanase by *Trichoderma longibrachiatum* on a mixture of wheat bran and wheat straw: optimization of culture conditions by Taguchi method. Enzym. Microb. Technol. 40, 801–805. https://doi.org/10.1016/j.enzmictec.2006.06.013.

Bagewadi, Z.K., Ninnekar, H.Z., 2015. Production, purifiation and characterization of endoglucanase from *Aspergillus fumigatus* and enzymatic hydrolysis of lignocellulosic waste. Int. J. Biotechnol. Biomed. Sci. 1, 25–32.

Bernardi, A.V., Gerolamo, L.E., Dinamarco, T.M., Tapia Blacido, D.R, 2023. Optimization of biomass saccharification processes with experimental design tools for 2G ethanol production: a review. Biofuels Bioprod. Biorefin. 17 (6), 1789–1806.

Bisaria, R., Vasudevan, P., Bisaria, V.S., 1990. Utilization of spent agro-residues from mushroom cultivation for biogas production. Appl. Microbiol. Biotechnol. 33, 607–609. https://doi.org/10.1007/BF00172560.

Brar, K.K., Agrawal, D., Chadha, B.S., Lee, H., 2019. Evaluating novel fungal secretomes for efficient saccharification and fermentation of composite sugars derived from hydrolysate and molasses into ethanol. Bioresour. Technol. 273, 114–121.

Campos, A.O., Asevedo, E.A., Souza Filho, P.F., Santos, E.S.D., 2024. Extraction of cellulases produced through solid-state fermentation by Trichoderma reesei CCT-2768 using green coconut fibers pretreated by steam explosion combined with alkali. Biomass 4 (1), 92–106.

Castilho, L.R., Medronho, R.A., Alves, T.L.M., 2000. Production and extraction of pectinases obtained by solid state fermentation of agro-industrial residues with

Aspergillus niger. Bioresour. Technol. 71, 45–50. https://doi.org/10.1016/S0960-8524(99)00058-9.
Chandra, M.S., Reddy, B.R., 2013. Exoglucanase production by *Aspergillus niger* grown on wheat bran. Ann. Microbiol 63, 871–877. https://doi.org/10.1007/s13213-012-0538-0.
Chandra, M.S., Sivaprasad, B.V., Riazunnisa, K., Chandrasekhar, T., 2022. Influence of carbon source and inoculum size on production of cellulolytic enzymes by a local isolate of *Aspergillus niger* on wheat bran. Adv. Biores. 13 (6), 199–208.
Chandra, M.S., Viswanath, B., Rajasekhar Reddy, B., 2010. Optimization of extraction of β-endoglucanase from the fermented bran of *Aspergillus niger*. Ind. J. Microbiol. 50, 122–126. https://doi.org/10.1007/s12088-010-0020-2.
Chandra, M.S., Viswanath, B., Reddy, B.R., 2007. Cellulolytic enzymes on lignocellulosic substrates in solid state fermentation by *Aspergillus niger*. Indian J. Microbiol. 47, 323–328.
Chandra, S., Rajasekhar, B.R., Choi, Y.-L., 2008. Optimization of extraction of Filter paperase from the fermented bran of *Aspergillus niger* in solid state fermentation. J Korean Soc. Appl. Biol. Chem. 51, 155–159.
Darabzadeh, N., Hamidi-Esfahani, Z., Hejazi, P., 2019. Optimization of cellulase production under solid-state fermentation by a new mutant strain of Trichoderma reesei. Food Sci. Nutr. 7 (2), 572–578.
Dey, P., Rangarajan, V., Singh, J., Nayak, J., Dilip, K.J., 2021. Current perspective on improved fermentative production and purification of fungal cellulases for successful biorefinery applications: a brief review. BioMass Convers. Biorefin. 1–29.
Dharmesh, A.N., Nikhil, S.B., Hasmukh, A.M., 2015. Optimization of upstream and downstream process parameters for cellulase-poor-thermosolvent-stable xylanase production and extraction by *Aspergillus tubingensis* FDHN1. Bioresour. Bioproces 2, 1–14. https://doi.org/10.1186/s40643-014-0029-1.
Ezeilo, U.R., Lee, C.T., Huyop, F., Zakaria, I.I., Wahab, R.A., 2019. Raw oil palm frond leaves as cost-effective substrate for cellulase and xylanase productions by *Trichoderma asperellum* UC1 under solid-state fermentation. J. Environ. Manage. 243, 206–217.
Fadel, M., 2000. Production physiology of cellulases and β-glucosidase enzymes of *Aspergillus niger* grown under solid state fermentation conditions. Online J. Biol. Sci. 1 (2000), 401–411.
Fernandez Lahore, H.M., Fraile, E.R., Cascone, O., 1998. Acid protease recovery from a solid-state fermentation system. J. Biotechnol. 62, 83–93.
Ghildyal, N.P., Ramakrishna, M., Lonsane, B.K., Karanth, N.G., 1991. Efficient and simple extraction of mouldy bran in a pulsed column extractor for recovery of amyloglucosidase in concentrated form. Process. Biochem. 26, 235–241. https://doi.org/10.1016/0032-9592(91)85005-9.
Giddel, M.R., Jivan, A.P., 2007. Waste to wealth, potential of rice husk in India a literature review. In: International Conference on Cleaner Technologies and Environmental Management PEC. Pondicherry, India, 2, pp. 4–6.
Kaab, A., Mohammad, S., Mobli, H., Nabavi-Pelesaraei, A., 2019. Use of optimization techniques for energy use efficiency and environmental life cycle assessment modification in sugarcane production. Energy 181, 1298–1320.
Kumar, V., Ahluwalia, V., Saran, S., Kumar, J., Patel, A.K., Singhania, R.R., 2021. Recent developments on solid-state fermentation for production of microbial secondary metabolites: challenges and solutions. Bioresour. Technol. 323, 124566.
Mandels, M., Weber, J., 1969. The Production of Cellulases, pp. 391–414.
Marin, M., Sanchez, A., Artola, A., 2019. Production and recovery of cellulases through solid-state fermentation of selected lignocellulosic wastes. J. Clean. Prod. 209, 937–946.
Megharaj, M., Kookana, K., Singleton, S., 1999. Activities of fenamiphos on native algal population and some enzyme activities in soil. Soil. Biol. Biochem. 31, 1549–1553. https://doi.org/10.1016/S0038-0717(99)00078-4.
Obeng, E.M., Wei, C.Y., SN, Adam, Ongkudon, C.M., 2020. Additives for cellulase enhancement. Borneo Int. J. Biotechnol. 1, 1–8.
Pal, A., Khanum, F., 2010. Production and extraction optimization of xylanase from *Aspergillus niger* DFR5 through solid state fermentation. Bioresour. Technol. 101, 7563–7569. https://doi.org/10.1016/j.biortech.2010.04.033.
Palit, S., Banerjee, R., 2001. Optimization of extraction parameters for recovery of amylase from the fermented bran of *Bacillus circulans* GRS313. Braz. Arch. Biol. 44, 107–111. https://doi.org/10.1590/S1516-89132001000100015.
Pandey, A., Selvakumar, P., Soccol, C.R., Nigam, P., 1999. Solid state fermentation for the production of industrial enzymes. Curr. Sci. 77 (1), 149–162.
Pandey, A.K., Negi, S., 2015. Impact of surfactant assisted acid and alkali pretreatment on lignocellulosic structure of pine foliage and optimization of its saccharification parameters using response surface methodology. Bioresour. Technol. 192, 115–125.
Pandey, A.K., Negi, S., 2020. Enhanced cellulase recovery in SSF from *Rhizopus oryzae* SN5 and immobilization for multi-batch saccharification of carboxymethylcellulose. Biocatal. Agric. Biotechnol. 26, 101656.
Pandya, J.J., Gupte, A., 2012. Production of xylanase under solid-state fermentation by *Aspergillus tubingensis* JP-1 and its application. Bioprocess. Biosyst. Eng. 35 (5), 769–779.
Pirota, R., Delabona, P., Farinas, 2013. Enzymatic hydrolysis of sugarcane bagasse using enzyme extract and whole solid-state fermentation medium of two newly isolated strains of *Aspergillus oryzae*. Chem. Eng. Trans. 38, 259–264. https://doi.org/10.3303/CET1438044.
Pragya, Krishna Kant S, Kumar, Sandeep, Manisha, Davender Singh, Kumar, Vijay, Singh, Bijender, 2023. Enhanced production and immobilization of phytase from *Aspergillus oryzae*: a safe and ideal food supplement for improving nutrition. Lett. Appl. Microbiol. 76 (2) https://doi.org/10.1093/lambio/ovac077.
Ranjan, R., Rai, R., Bhatt, S.B., Dhar, P., 2023. Technological road map of Cellulase: a comprehensive outlook to structural, computational, and industrial applications. Biochem. Eng. J. 28, 109020.
Sadh, P.K., Duhan, S., Duhan, J.S., 2018. Agro industrial wastes and their utilization using solid state fermentation: a Review. Bioresour. Bioprocess. 5, 15.
Santos, D.A., Oliveira, M.M., Curvelo, A.A., Fonseca, L.P., Porto, A.L., 2017. Hydrolysis of cellulose from sugarcane bagasse by cellulases from marine-derived fungi strains. Int. Biodeterior. Biodegradation 121, 66–78.
Sarangi, M., Bhattacharyya, S., Behera, R.C., 2009. Effect of temperature on morphology and phase transformations of nano-crystalline silica obtained from rice husk. Phase Transit. 82 (5), 377–386.
Shruthi, K., Suresh Yadav, P., Siva Prasad, B.V., Chandra, M.S., 2018. Production of cellulase by a local isolate of *Aspergillus unguis* on different lignocellulosic substrates in solid state fermentation. J. For. Res. 30 (1), 205–212. https://doi.org/10.1007/s11676-018-0619-4.
Shyamsundar, P., Springer, N.P., Tallis, H., Polasky, S., Jat, M.L., Sidhu, H.S., Krishnapriya, P.P., Skiba, N., Ginn, W., Ahuja, V., Cummins, J., 2019. Fields on fire: alternatives to crop residue burning in India. Science 365 (6453), 536–538.
Singh, M., Tiwari, D.P., Kumar, A., Kumar, R., 2003. Effect of feeding transgenic cottonseed vis-a-vis non-transgenic cottonseed on haematobiochemical constituents in lactating murrah buffaloes. Asian Australas. J. Anim. Sci. 16, 1732–1737.
Singh, Y., Sidhu, H.S., 2014. Management of cereal crop residues for sustainable rice-wheat production system in the Indo-Gangetic plains of India. Proc. Indian Natl. Sci. Acad. 80 (1), 95–114.
Slayton, T., Timmer, C.P., 2008. Japan, China and Thailand can solve the rice crisis—But US Leadership is needed. Center Glob. Dev. Notes. *Accessed August., 22*.
Sudiyani, Y., Muryanto, 2012. The potential of biomass waste feedstock for bioethanol production. In: Proceeding of International Conference on Sustainable Energy Engineering and Application, pp. 5–10.
Tripathi, M.K., Mishra, A.S., 2007. Gulcosinolates in animal nutrition: a Review. Anim. Feed Sci. Technol. 132, 1–27.
Tunga, R., Banerjee, R., Bhattacharya, B.C., 1999. Some studies on optimization of extraction process for protease for production in SSF. Bioprocess. Eng. 20, 485–489. https://doi.org/10.1007/s004490050619.
Wallheimer, B. and Brian, Y. (2010). Rice hulls a sustainable drainage option for greenhouse growers. *Purdue University-2012*.
Yadav, P.S., Prasad, Siva, B.V, Chandra, M.S, Maddela, N.R, Prasad R, 2024. Leaching approach for β-glucosidase extraction from fermented rice husk in solid state cultivation by *Aspergillus protuberus*. Curr. Microbiol. 15 (6), 140. https://doi.org/10.1007/s00284-024-03641-9, 81.
Yadav, P.S., Shruthi, K., Prasad, B.S., Chandra, M.S., 2016. Enhanced production of β-glucosidase by new strain *Aspergillus protuberus* on solid state fermentation in rice husk. Int. J. Curr. Microbiol. App. Sci. 5 (12), 551–564.
Yadav, P.S., Shruthi, K., Siva Prasad, B.V., Chandra, S.M., 2017. Isolation and identification of *Aspergillus protuberus* from Mahanandi forest sample and investigation of its cellulase production. Indian J. Adv. Chem. Sci. 5 (1), 8–15.
Zhu, J.Y., Subhosh Chandra, M., Gu, F., Gleisner, R., Reiner, R., Sessions, J., Marrs, G., Gao, Jo., Anderson, D., 2015. Using sulfite chemistry for robust bioconversion of Douglas-fir forest residue to bioethanol at high titer and lignosulfonate: a Pilot-Scale Evaluation. Bioresour. Technol. 179, 390–397.