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Bioconversion of Some Agro-Residues into Organic Acids by Cellulolytic Rock-Phosphate-Solubilizing *Aspergillus japonicus*

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Abstract: Biological-based conversion of agricultural residues into bioactive compounds may be considered to be the basis for various vital industries. However, finding a suitable microorganism is a challenge in the bioconversion process. Therefore, this study was conducted to find local fungal isolates able to convert a combination of plant biomass residues into organic acids (OAs). Based on their cellulase and phytase activities and rock phosphate (RP) solubilization potential, an efficient 15 fungal isolates (named F1 to F15) were selected and identified by both morphological and molecular methods using the 18S rRNA sequencing technique. The best fungal isolate (F15) was identified as *Aspergillus japonicus*. After 4 weeks of incubation below solid-state fermentation (SSF) with a mix of sugarcane bagasse and faba bean straw (3:7), with 7.5% (*v*/*w*) fungal inoculum to the growth medium, the biodegradation process by the fungus reached its peak, i.e., maximum cellulolytic activity and RP solubilization ability. Under such fermentation conditions, seven organic acids were detected using HPLC, in the following order: ascorbic acid > oxalic acid > formic acid > malic acid > succinic acid > lactic acid > citric acid. Based on the results, *Aspergillus japonicus* (F15) could produce OAs and cellulose enzymes, and could be considered a new single-step bio-converter of sugarcane bagasse and faba bean straw residues into OAs. Furthermore, this fungus could be a new source of fungal cellulose, and could present a practical approach to reducing environmental contamination. Additional work is encouraged for more optimization of fermentation conditions.

Keywords: rock phosphate solubilization; cellulases; sugarcane bagasse; faba bean straw; phytase

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1. Introduction

Microbial bioconversion of agro-residues into cellulosic biomass is a prominent source of renewable, cheap, and unexploited energy [1,2]. The bio-residues include stems, leaves, corn kernels, bagasse, woody crops, and forest residues. Moreover, many lignocellulose residues can be produced primarily from the byproducts of industrial and agricultural processes, including municipal solid residues, citrus peel residues, sawdust, paper pulp, paper mill sludge, and kitchen waste [3,4].

Bioconversion of biomass has important advantages over other alternative energy strategies, because biomass is the most abundant and the most renewable biomaterial on our planet. Bioconversion of lignocellulosic residues is initiated primarily by microorganisms such as fungi and bacteria that are capable of degrading lignocellulolytic materials.

Fungi such as *Trichoderma reesei* and *Aspergillus niger* produce large amounts of extracellular cellulolytic enzymes, whereas bacteria and a few anaerobic fungal strains mostly produce cellulolytic enzymes in a complex called a cellulosome, which is associated with the cell wall. In filamentous fungi, cellulolytic enzymes such as endoglucanases, cellobiohydrolases (exoglucanases), and β-glucosidases work efficiently on cellulolytic residues in a synergistic manner. In addition to cellulolytic/hemicellulolytic activities, higher fungi such as basidiomycetes (e.g., *Phanerochaete chrysosporium*) have unique oxidative systems that, together with ligninolytic enzymes, are responsible for lignocellulose degradation [5]. *Aspergillus japonicus* can release soluble phosphate from rock phosphate (RP) and excrete multiple organic acids; the primary mechanism of RP solubilization is the production of organic acids [6]. Furthermore, Nasr et al. [7] discovered that *A. japonicus* was the most capable fungus in solubilizing phosphate rock, producing various organic acids (i.e., salicylic, ascorbic, citric, formic, lactic, oxalic, and malic acids) during this process. On the other hand, Atallah and Yassin [8] found that *Aspergillus japonicus* produces a high content of oxalic acid compared to other *Aspergillus* spp., suppressing the growth of *Sclerotinia sclerotiorum*.

About 50% of carbon globally fixed by photosynthesis is found in the lignocelluloses of plant cell walls. Lignocelluloses are abundant, and are possible substrates for bioconversion. Lignocellulosic components comprise three major components (i.e., hemicellulose, cellulose, and lignin) of plants' cell walls, and have high proportions in biomass crops [9].

Renewable resources have become an important raw material for industries' green and sustainable development as a substitute for petrochemical resources. Lignocellulose resources, which are abundant in hemicellulose, lignin, and cellulose, are the most widely distributed and widespread renewable resources on Earth. Bagasse, a cellulose residue obtained after crushing and pressing sugarcane juice, is an essential renewable biomass resource. Sugarcane is an annual crop, and its fiber morphology is closer to that of wood fiber than that of other grass fibers. Therefore, bagasse is considered to be an ideal fiber material, of which cellulose accounts for approximately 46–55% [10]. Moreover, large quantities of agricultural yield residues are burned in open fields, particularly in production areas with a high population density, developed economic zones, and affluent scorbutic fuel preparation [11–13].

Several industrial waste products, due to their organic and nutrient-rich composition, have been utilized as resources for the production of value-added products such as organic acids, biofuels, biopesticides, biohydrogen, enzymes, and bioplastics via microbial fermentation processes [14].

The cultivated area of sugarcane and faba bean plants in 2020 was 136,500 and 27,813 hectares, respectively, based on data from the Ministry of Agriculture and Land Reclamation, and these crops generated large amounts of agro-residues after harvest processes; the productivity of an acre of sugar cane and faba bean crops is estimated at 2 tons, respectively, which means that the total production of sugar cane is approximately 650,000 tons. The municipal bean crop is around 132,442 tons, and the amount of waste generated is estimated at one-third of the produced quantity. Sugar cane bagasse is estimated at 216,667 tons, and faba bean straw at 44,147 tons. Therefore, new technology must reuse these agricultural residues in an environmentally friendly manner to deal with residues readily and significantly—primarily to protect plants from harmful disease vectors [1,15,16]. The value of the global organic acid market stood at USD 6.94 billion in 2016, and is projected to reach USD 12.54 billion by the end of 2026. Organic acids constitute a significant portion of the global fermentation market, and microbiological production is an economic alternative using pretreated agricultural waste [17].

Lignocellulosic biomass is degraded through the synergistic effects of three cellulase complex enzymes, including endoglucanases (endo-1, 4-β-glucanases, EC 3.2.1.4), cellobiohydrolases (exo-1, 4-glucanases, EC 3.2.1.91), and beta-glycosides (-D-glucoside glucohydrolases, EC 3.2.1.21) [18,19]. Using microorganisms is a promising method for breaking down lignocellulose through bioconversion to microbial biomass as a nutritional supplement, improved by the cellulosic and hemicellulosic components of agricultural

residues. In this context, cellulolytic fungi play a critical role in natural biodegradation by producing cellulolytic enzymes, and these enzymes have been applied in different technological industries [20–22]. Some *Aspergillus* spp. are considered to be a reservoir for the manufacture of OAs [23].

Yeasts and fungi can convert the simpler carbohydrates such as sugars and malic acid in grape and apple pomace to ethanol and high-value carboxylic acids, such as lactic, fumaric, succinic, and citric acids [24]. *Aspergillus* is one of the most important industrial filamentous fungal species; it is nontoxic, safe for production, and offers several advantages, such as higher productivity, higher yields, and lower contamination risk. Thus, these fungal isolates are utilized. Filamentous fungi of the genus *Aspergillus* are crucial for the generation of bio-based OAs. Due to their exceptional ability to secrete substantial volumes of desirable OAs, a variety of *Aspergillus* strains derived from phylogenetically distantly correlated spp. have been effectively employed in the industrial manufacture of OAs. Several decades have been spent trying to uncover the procedures of OAs' biosynthesis in various *Aspergillus* spp., and using genetic engineering to increase the production of preferred OAs. The development in genetic engineering of aspergilli for the generation of OAs is highlighted in this study, which outlines recent advancements in the basic knowledge of physiological aspects of OAs' production by fungal biocatalysts. The difficulties of using aspergilli as commercial cell factories for the manufacture of OAs in the future are also explored.

Solid-state fermentation (SSF) is the multiplication of microbes on the surface and the interior of a solid support (i.e., inert support or substrate), not including any free-running water. The typical microbial flora used in SSF are filamentous fungi, because of their hyphal growth and higher production of enzymes and byproducts compared to other techniques [25,26]. Moreover, the SSF technique has many advantages, such as the low volume of liquid required to recover of product. Thus, it can lower the cost of final manufacturing and consequent residue processing, increase the availability of simple and inexpensive nutrients of fermentation, reduce power requirements, and usually produce higher yields, because the absence of free-flowing water in the pollutants prevents their growth, reducing the risk of pollution [27,28]. Thus, this work was designed to obtain efficient cellulolytic fungi of the *Aspergillus* genus for the biodegradation of biowaste materials—i.e., sugarcane bagasse (SB) and faba bean straw (FBS)—under SSF by filamentous fungi for the production of OAs.

2. Materials and Methods

2.1. Substrates

The agro-residues—sugarcane bagasse (SCB) and faba bean straw (FBS)—were dried at 70 °C and ground in an electric mill. The chemical analysis of these residues was conducted as described by Westerman [29], and the results are summarized in Table 1. The rock phosphate used in the current study was (RP, 7.97%).

Table 1. Chemical analysis of the two agro-residues used in this study.

Parameters *	Sugarcane Bagasse	Faba Bean Straw
Organic carbon (%)	56.00 ± 0.12	44.00 ± 0.34
Total N (%)	0.70 ± 0.22	1.47 ± 0.11
C/N ratio	80.00 ± 0.14	29.93 ± 0.35
Total P (%)	0.23 ± 0.09	0.32 ± 0.07
Total K (%)	0.79 ± 0.16	1.34 ± 0.08

* Each presented value for all parameters represents the mean (± SD) of three replications of the experiment.

2.2. Isolation of Fungal Isolates from Sugarcane Bagasse and Faba Bean Straw

Samples of decayed sugarcane bagasse (SCB) and faba bean straw (FBS) were crushed, suspended in saline solution (NaCl 8.5 g/L), and then stirred for 10 min. One milliliter of the suspension was added to microcrystalline cellulose agar (Sigma-Aldrich, St. Louis, MO, USA) and kept at 28 °C for five days [30]. The pure colonies on the plate with surrounding

clear zones were collected for isolation, selection due to their characteristics, and primary identification according to Abo-State [31]. The fungi were maintained on potato dextrose agar (PDA: M069, MIMEDIA, Mumbi, India).

2.3. Inoculum Preparation

The 15 fungal isolates were selected based on their growth on microcrystalline cellulose agar. Each isolate was cultured on plates of PDA and kept for one week at 28 °C. The fungal isolates were enveloped with sterile distilled water (10 mL for each isolate), and suspensions were prepared for inoculation. The inoculum for further studies was adjusted with sterilized distilled water to 1.8×10^8 spores/mL for inoculation.

2.4. Screening of Fungal Isolates

The fungal isolates were screened for cellulase production from the isolation trial using 100 mL of potato dextrose (PD) medium adjusted to pH 5 in Erlenmeyer flasks (500 mL). The flasks were inoculated with 1 mL of inoculum containing 1.8×10^8 spores/mL. The inoculated flasks were kept at 28 °C for 5 days on a rotary shaker at 150 rpm. After the incubation time, the biomass of the fungi was extracted by filtration. The obtained filter sheet was dried at 70 °C in an oven until the weight became stable, and was then weighed. The multiplication of fungi was detected in terms of the dry weight (DW) of mycelium (mg/flask) [32]. Otherwise, the filtrate was applied as rough enzyme (RE) accommodation to evaluate cellulase activity (CMCase, FPase and β-glucosidase).

2.5. Cellulase Activity Test

The cellulase activity was measured by incubating 1.0 mL of fungal growth filtrate containing RE and a 1.0 mL buffer of 0.1 M citrate (pH 4.8) with 1% salicin, CMC, and 50 mg of powdered Whatman No. 1 filter paper as substrates (CF) for 20, 40, and 60 min, respectively, at 50 °C. Cellulase activities were estimated by calculating the reduction in sugar levels using the dinitrosalicylic acid method, as described by Ghose [33]. One unit of enzyme activity was identified as the quantity of enzyme that produced 1 µmol of glucose per minute. β-Glucosidase and CMCase assays were performed as described by Miller [34], and the filter paper assay was performed as described by Ghose [33]. A set of reference standard solutions and curves were prepared for each estimation and determined for calculation.

2.6. Rock Phosphate Solubilization Assay

The more active cellulolytic fungal isolates in terms of their rock phosphate (RP) solubilization efficacy were selected using the liquid medium described by Nautiyal [35]. Erlenmeyer flasks (500 mL) containing 100 mL of broth medium (gL^{-1})—glucose, 10; $(NH_4)_2SO_4$, 0.1; $MgSO_4$. 7 H_2O, 0.25; KCl, 0.2; $MgCl_2$, 0.6; H_2O, 5.0—and 25 mg P_2O_5 as the source of P (rock phosphate) were inoculated with 5% (v/v) of each isolate and kept at 28 °C on a rotary shaker at 150 rpm for one week. The pH, titratable acidity (TA), soluble P, and phytase activity were measured in the supernatants obtained by centrifugation at 4000 rpm for 5 min, as described by Cerezine et al. [36], Westerman [29], and El-Sawah et al. [37]. The phytase activity was identified as the quantity of enzyme that produced 1 µmol of inorganic phosphorus mL^{-1} min^{-1}.

2.7. Detection of the Best Fungal Isolates

The isolates were identified for the genus *Aspergillus* based on morphological and phenotypic criteria defined by several authors [38–41]. The best isolates were determined based on 18S rRNA gene sequence analysis. Sequencing was performed using an automated DNA sequencer (ABI Prism 3130 Genetic Analyzer by Applied Biosystems Hitachi, Chiyoda, Tokyo, Japan). Genomic DNA was obtained by the hexadecyltrimethylammonium bromide (CTAB) technique, and the integrity and level of purified DNA were established by agarose gel electrophoresis [42]. The DNA level was customized to 20 ng/µL for PCR amplification.

The forward primer used with the isolates was (5\AGA GTT TGA TCC TGG CTC AG 3\), and the reverse was (5\ GGT TAC CTT GTT ACG ACT T 3\). PCR products were isolated by electrophoresis on 1.5% agarose gels stained with ethidium bromide and documented using the AlphaImager TM1200 documentation and analysis system. The obtained polymorphic differences were analyzed via the program NTSYS-PC2 by assessing the distance of isolates using Jaccard's similarity coefficient. The recovered 18S rRNA of the isolates was compared to the sequences in the NCBI GenBank database using the Basic Alignment Search Tool (BLAST) and inferring the tree's topology via the clustering method UPGM. The sequences were registered in the NCBI database.

2.8. Solid-State Fermentation (SSF)

As described by Xu et al. [43], the hydrolysis of various substrates via an SSF method was performed in 500 mL Erlenmeyer flasks. About 20 g of each substrate's powder was taken in individual flasks, and 30 mL of mineral salt solution ((g L^{-1}): 4.0 KH_2PO_4, 1.6 $(NH_4)_2SO_4$, and 1.0 $MgSO_4$) was added as a moistening agent. Before autoclaving, 100 mg of P_2O_5 as a source of P (rock phosphate) was added individually to each flask. The substrate was inoculated with 5% inoculum from the spore suspension containing ~1.4×10^8 spores mL^{-1} [44]. The starting moisture content was adjusted to 65% and kept at 28 °C for 9 days under static conditions, and at the end of the fermentation time, 0.05 M citrate buffer (pH 4.8) was added to give a total of 50 mL/flask. The flasks were shaken at 150 rpm for 30 min, filtered via Whatman No.1 filter paper, and then centrifuged for 10 min at 4000 rpm to obtain a clear filtrate. The filtrate was tested for both cellulase enzymes and soluble P. Additionally, effects of the time course (0–35 days), five concentrations of P (0.0, 25, 50, 75, and 100 mg P_2O_5 from RP), and 4 inoculum ratios (2.5, 5.0, 7.5, and 10%, *v*/*w*) were studied under different ratios of SCB and FBS residue mixtures using the best selected fungal isolate.

2.9. Synthesis of Organic Acids

For further optimization trials with SSF, the optimal fermentation conditions were implemented for the production of OAs, and remnants of the fermentation process were dried in an oven at 80 °C to constant weight to estimate residual DW. Furthermore, the extracts from the decomposition process were applied to measure pH, TA, P, and cellulase activity, as described above. The contents of OAs were determined by high-performance liquid chromatography.

2.10. High-Performance Liquid Chromatography (HPLC)

The quantities and levels of the produced OAs in the hydrolysates were assessed via HPLC. Ascorbic, citric, formic, lactic, maleic, oxalic, and succinic acids were assessed by HPLC using the "Win Chrome Chromatography Version 1.3 software PerkinElmer, USA, GBC U.V/Vis Detector and GBC L.C 1110 Pump" HPLC instrument, and a 100 × 4.6 mm KROMASIL column with a mobile phase of 0.2 M H_2PO_4: methanol (90:10) (0.72:0.08), at a flow rate of 1mL/min. Recognition was recorded by UV absorbance at 254 nm. The OAs were quantified by reference to the peak areas and the retention periods for each OA. The HPLC technique was satisfactory (Andersson and Hedlund, 1983). Organic acid analysis was performed via HPLC (Agilent 1100 HPLC system), using a Hyper REZXP Carbohydrate H 8 µm column (300 × 7.7 mm) with a mobile phase of 5 mM H_2SO_4, a flow rate of 0.6 mL/min, and a column temperature of 55 °C.

The injection volume of the samples applied for HPLC analysis was 20 µL of the final extract. Detection was performed by UV absorbance at 280 nm. The acetic acid was detected and quantified using an Atlantis™ dC18 column (2.1 mm × 150 mm) with a particle size of 5 µm. Two mobile phases were employed to produce a linear gradient with a 0.2 mL/min flow rate. Mobile phase A was water with 0.01% (*v*/*v*) formic acid, and mobile phase B was acetonitrile with 0.01% (*v*/*v*) formic acid. The linear gradient was from 0% to 10% B for the first 12 min, and from 10% to 50% B for the next 3 min; mobile phase B was kept at 50% for

3 min, and then lowered to 0% for 1 min. The temperature of the column was maintained at 28 °C, and the injection volume was 5 µL. The OAs were quantified by reference to the peak areas and retention periods achieved, compared to the authentic standards for the seven OAs in in the mobile phase [45]. The process of bioconversion of some agro-residues into organic acids by *Aspergillus japonicus* is presented in Figure 1.

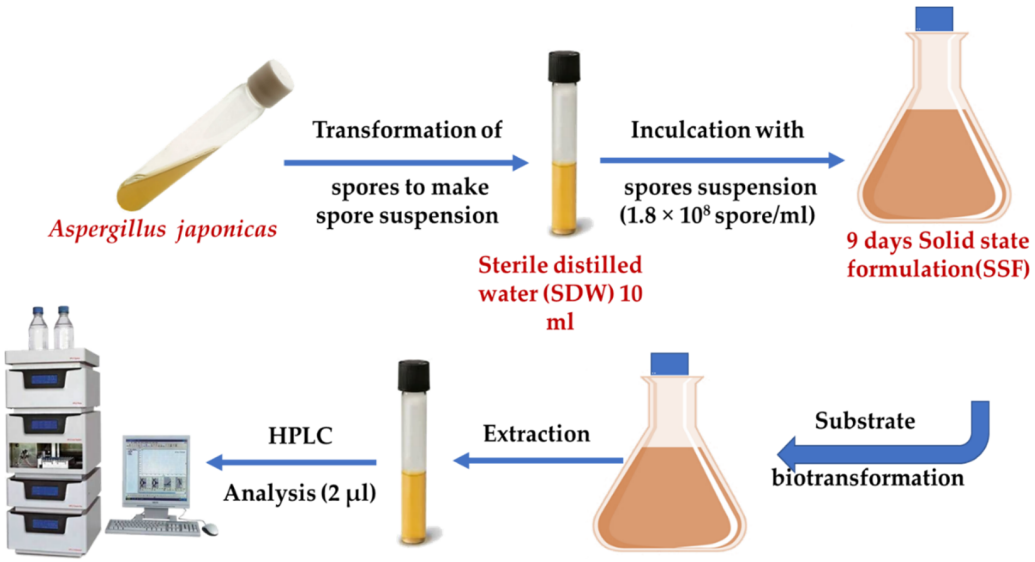

Figure 1. A graphical presentation of the bioconversion of some agro-residues into organic acids by *Aspergillus japonicus*.

2.11. Statistical Analysis

The trials were carried out in independent biological triplicates, and the data are presented as mean values ± standard deviation. SAS was used to carry out all of the statistical approaches. Tukey's test ($p < 0.05$) was used to assess all data.

3. Results and Discussion

3.1. Identification of Cellulase Production and RP Solubilization by Fungal Isolates

Fifteen fungal isolates, which showed the maximum cellulolytic activity on agar plates, were recovered from SCB (10 isolates) and FBS (5 isolates) residues. These fungi were selected preliminarily based on the distinguished characteristics of their colonies and their microscopic morphology (Figure 2). After growth of the fungal isolates on malt extract 20% sucrose agar (MEA) [46], microscopic observations were carried out on conidiophores produced on MEA after 7–10 days. These belonged to 15 fungal spp. of the genus *Aspergillus*, as follows: *Aspergillus japonicus* (10 isolates, named F1, F2, F3, F4, F5, F6, F7, F8, F9, and F15), and *A. niger* (5 isolates, named F10, F11, F12, F13, and F14). The colony morphology of *Aspergillus* sp. showed a black-colored colony, and the microscopic photograph showed the arrangement of the conidia.

The genus *Aspergillus* is characterized by the formation of flask-shaped or cylindrical phialides in either a single or double series on the surface of a vesicle at the apex of a conidiophore [47]. *Conidia* are deciduous and globose, oblong-to-elliptical in shape, and present various colors. In 1985, Gams et al. revised the groups and assigned them to 18 sections as a formal taxonomic status [48]. Currently, there are approximately 250 species assigned to 17 sections in the family Aspergillaceae [46], and this number will continue to grow as new species are described. Filamentous fungi from the genus *Aspergillus* are of high importance for bio-based organic acid production. To date, a number of *Aspergillus* strains belonging to phylogenetically distantly related species have been successfully applied in the industrial production of organic acids, due to their excellent capabilities of secreting high amounts of the desired organic acids [49]. *Aspergillus* is a diverse genus containing many species recorded as efficient organic acid producers. In general, the reported aspergilli for

organic acid production can be divided into two major groups: industrial workhorses, and species with potential for organic acid production. The classification and identification of *Aspergillus* species has been based on phenotypic characteristics, but in recent decades has been strongly influenced by molecular and chemotaxonomic characterization. Thus, the fungal isolates (F5, F11, F14, and F15) were selected for identification based on molecular documentation using 18S rRNA gene sequencing.

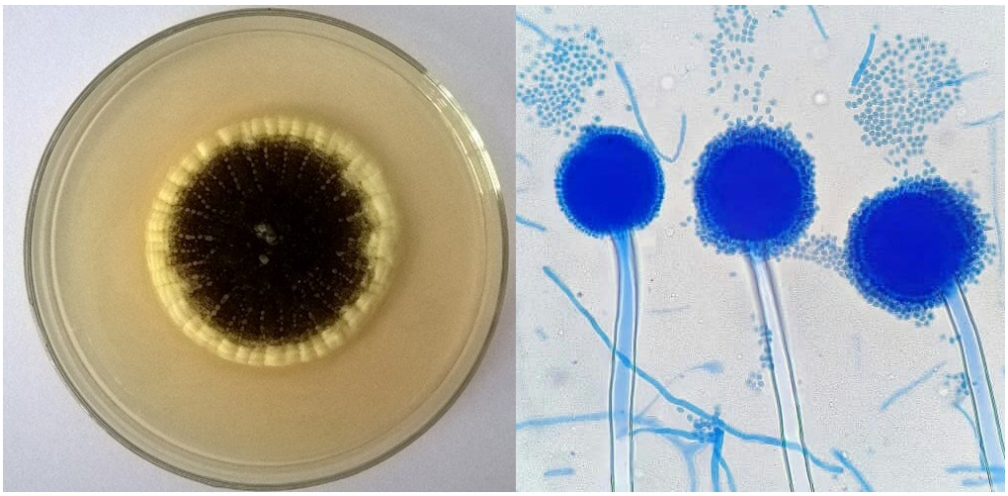

Figure 2. Images of *Aspergillus japonicus* in the dish, as well as pictures of the spores under a light microscope at 400× magnification.

The recovered sequences of 550 bp of amplified 18S rRNA fragments on agarose gel revealed a resemblance of the target sequences to the closely related fungal sequences of *Aspergillus* spp. It is a fact that the *Aspergillus* spp. F5, F11, F14, and F15 are highly similar to *Aspergillus japonicus*. The molecular characterization of the cellulolytic RP-solubilizing *Aspergillus japonicus* isolate named SM was performed based on 18S rRNA gene sequencing. This sequence was submitted to GenBank with the accession number MN960315, and aligned with other 18S rRNA sequences of *Aspergillus* spp. obtained from the GenBank databases by BLAST searches, showing 99.76% homology with those of many species of the genus *Aspergillus.* The phylogenetic correlation between the strain SM and other correlated fungi, on the basis of 18S rRNA gene sequencing of members of the genus *Aspergillus*, was determined (Figure 3) following the procedure of neighbor-joining. The tree appeared to show a close phylogenetic association of the strain SM with certain other *Aspergillus* spp. Phylogenetic analysis suggested that the strain SM consistently falls into a clade together with *A. japonicus* strains (Figure 3). Thus, it is suggested that the strain SM should be included in the genus *Aspergillus* as *A. japonicus* strain SM (MN960315.1).

3.2. The Cellulosic Activity of the Isolated Fungi from SCB and FBS

Most of the isolated fungi revealed good growth and strong sporulation on the incubation media. Cellulase production was quantitatively determined on the broth media, and the activity of cellulase enzymes is shown in Figure 4. As mentioned, the cellulase activities of isolates F5, F11, F14, and F15 were higher than those of the other isolates. These isolates are valuable in the bioconversion of cellulosic residues. Nasr et al. [7] found that *A. japonicus* was more efficient at solubilizing insoluble phosphate than other fungal isolates during phosphate solubilization. This fungal strain produced more than six different OAs compared to *Penicillium* spp.

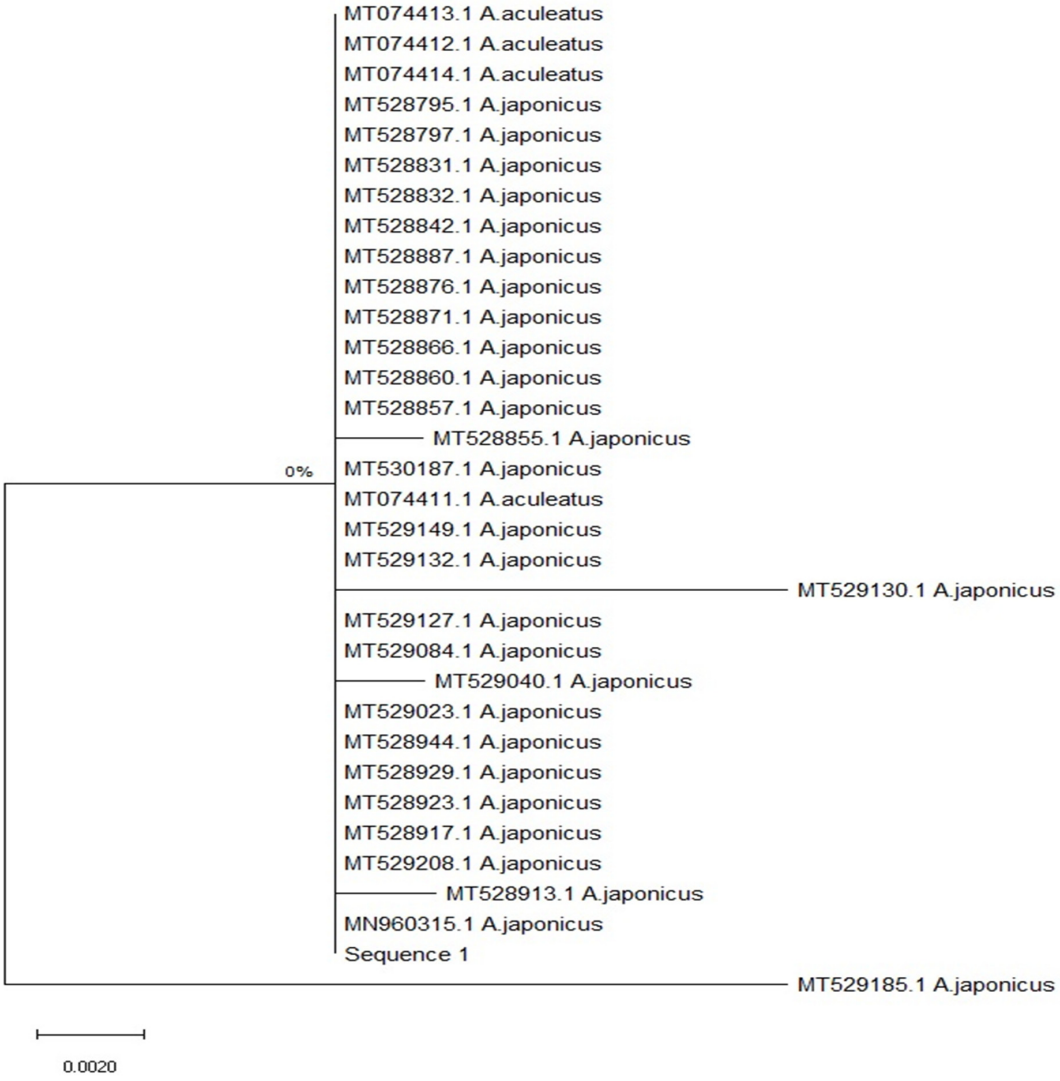

Figure 3. Molecular phylogenetic tree of the partial 18S rRNA sequence of *Aspergillus japonicus* MN960315.

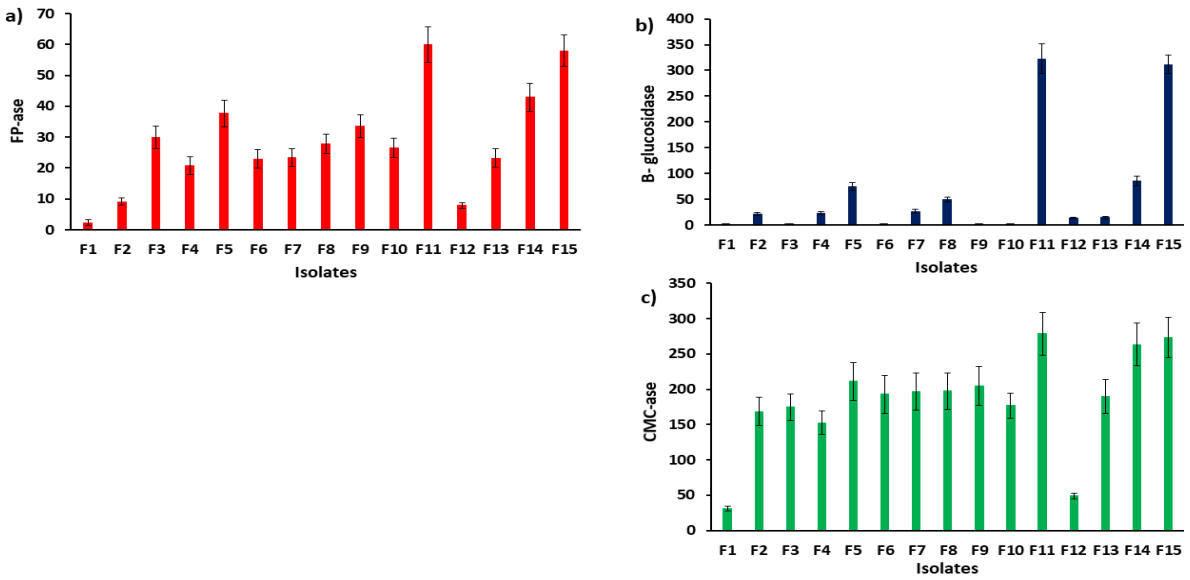

Figure 4. The activity of CMCase cellulases in the filtrates of different isolated fungi.

3.3. Rock Phosphate (RP) Solubilization by the Isolated Fungi

After cellulose production and rock phosphate solubilization (Figure 4 and Table 2), the fungal isolates recovered from the earlier assessment were further screened based on RP solubilization. Commonly, as shown in Table 2, all fungi reduced the final culture pH to the acidic side. Additionally, four isolates appeared to have phytase activity, and released soluble P from RP. In descending order, the amounts of soluble P produced were 30.151, 29.972, 29.605, and 29.091 μg mL^{-1} in the F15, F11, F14, and F5 culture filtrates, respectively, compared to the other isolates, meaning that these fungi have an effective system for RP solubilization.

Table 2. Bio-solubilization of rock phosphate by different *Aspergillus* spp.

Fungal Isolates	Final Culture pH *	Titratable Acidity (μg NaOH mL^{-1}) *	Soluble P (μg mL^{-1}) *	Phytase (U mL^{-1}) *
F1 (*Aspergillus* spp.)	5.19 ± 0.23 ^{a}	67.48 ± 0.23 ^{b,c}	24.47 ± 0.43 ^{b,d}	15.10 ± 0.23 ^{a,b}
F2 (*Aspergillus* spp.)	5.76 ± 0.13 ^{a}	74.88 ± 0.22 ^{a,b}	24.03 ± 0.54 ^{c,d}	12.15 ± 0.35 ^{e}
F3 (*Aspergillus* spp.)	5.57 ± 0.15 ^{a}	72.44 ± 0.32 ^{b}	24.95 ± 0.22 ^{b,c}	14.29 ± 0.41 ^{c,d}
F4 (*Aspergillus* spp.)	5.73 ± 0.23 ^{a}	74.54 ± 0.24 ^{a,b}	23.80 ± 0.12 ^{d}	15.29 ± 0.33 ^{c}
F5 (*Aspergillus* spp.)	4.82 ± 0.18 ^{b}	53.82 ± 0.27 ^{d}	29.09 ± 0.15 ^{a}	16.21 ± 0.21 ^{a,b}
F6 (*Aspergillus* spp.)	5.91 ± 0.14 ^{a}	76.88 ± 0.28 ^{a}	22.55 ± 0.17 ^{e}	14.23 ± 0.23 ^{d}
F7 (*Aspergillus* spp.)	5.95 ± 0.14 ^{a}	77.35 ± 0.23 ^{a}	21.11 ± 0.31 ^{e}	14.87 ± 0.11 ^{d}
F8 (*Aspergillus* spp.)	5.31 ± 0.18 ^{a}	69.09 ± 0.25 ^{b}	20.20 ± 0.32 ^{e,f}	14.00 ± 0.23 ^{d}
F9 (*Aspergillus* spp.)	5.79 ± 0.11 ^{a}	75.36 ± 0.24 ^{a}	19.22 ± 0.35 ^{f,g}	14.59 ± 0.28 ^{c}
F10 (*Aspergillus* spp.)	5.20 ± 0.09 ^{a}	67.63 ± 0.34 ^{b,c}	25.29 ± 0.37 ^{c}	15.02 ± 0.37 ^{b,c}
F11 (*Aspergillus* spp.)	4.41 ± 0.17 ^{b}	60.75 ± 0.29 ^{d}	29.97 ± 0.45 ^{a}	16.44 ± 0.55 ^{a}
F12 (*Aspergillus* spp.)	5.573 ± 0.13 ^{a}	72.44 ± 0.30 ^{a,b}	23.68 ± 0.44 ^{c,d}	15.29 ± 0.32 ^{b,c}
F13 (*Aspergillus* spp.)	5.932 ± 0.16 ^{a}	77.11 ± 0.33 ^{a}	24.79 ± 0.12 ^{b}	15.39 ± 0.31 ^{b,c}
F14 (*Aspergillus* spp.)	4.652 ± 0.18 ^{b}	57.33 ± 0.39 ^{e}	29.60 ± 0.23 ^{a}	16.33 ± 0.33 ^{b}
F15 (*Aspergillus* spp.)	4.330 ± 0.16 ^{b}	62.47 ± 0.35 ^{d,e}	30.15 ± 0.13 ^{a}	17.78 ± 0.36 ^{a}

* The data are presented as the mean ± SD of three replicates. The superscript lowercase letters within columns indicate significant differences in the rock-phosphate-solubilizing ability of the different isolates ($p < 0.05$).

3.4. Cellulase Synthesis and RP Solubilization by *A. japonicus* under SSF Conditions

After the earlier screening experiments and molecular detection, *A. japonicus* was grown under SSF conditions to optimize cellulase manufacturing and RP solubilization. For this purpose, five incubation periods (1–5 weeks), four levels of P (25–100 mg P_2O_5 from RP), and four inoculum ratios (2.5–10%, *v*/*w*) were studied under different ratios of SCB and FBS residues. The time-course profiles of FPase, CMCase, and β-glucosidase synthesis, as well as soluble P during SSF, appeared to be the same, and these enzymes increased under the same conditions with *A. japonicus* (Table 3). OF the different treatments, T4 (0.3 g SCB + 0.7 g FBS) recorded the best treatment for the production of the tested enzymes, which elevated dramatically in the first three weeks and achieved a maximum in the fourth week, before reducing in the fifth week. A parallel trend was noticed in the RP solubilization process.

The probable reason for increased enzyme activities is that the enzyme synthesis in different microorganisms achieved the highest levels in the log phase and declined during death. During the death phase, reduction in nutrient levels and cellular fragmentation are widespread, facilitating the liberation of intracellular materials and proteases in the fermentation broth [50,51]. Different results were reported by several investigators, including Liua and Orskovb [52], Badhan et al. [53], Saber et al. [54], El–Nahrawy et al. [55], and Narsale et al. [56]. Considering the incubation period, the time-course was selected as four weeks for subsequent studies.

The C/N ratio is significant for any fermentation process. A proper C/N ratio for pure culture is necessary to optimize aerobic fermentation from organic substrates under submerged conditions. It is therefore necessary to maintain proper composition of the feedstock for efficient scaling-up [57]. Moreover, at lower C/N ratios, during fermentation by *Aspergillus giganteus*, a filamentous form of growth was maintained, with the hyphae

being scarcely branched and without bulbous cells. Membrane perturbation due to the induction of intracellular oxidative stress was also noted at higher C/N ratios [58].

Table 3. Time-course profiles of cellulase production and P solubilization by *A. japonicus* under different ratios of sugarcane bagasse and faba bean straw residues.

Treatment	FPase (Unit g^{-1}) *	β-Glucosidase (Unit g^{-1}) *	CMCase (Unit g^{-1}) *	Soluble P (μg g^{-1}) *
		First week		
T1	22.34 ± 2.2	66.02 ± 6.50	166.29 ± 11.20	60.01 ± 4.30
T2	24.39 ± 3.2	71.17 ± 5.98	159.99 ± 10.12	66.07 ± 4.61
T3	25.75 ± 2.6	75.25 ± 6.01	166.97 ± 12.00	68.15 ± 4.88
T4	30.49 ± 2.9	90.47 ± 6.00	171.45 ± 13.65	70.07 ± 5.09
T5	29.30 ± 3.3	88.9 ± 5.50	167.39 ± 12.87	68.12 ± 5.12
T6	27.12 ± 2.4	80.36 ± 7.02	166.56 ± 9.23	60.16 ± 5.00
T7	26.39 ± 2.1	77.17 ± 6.22	162.88 ± 9.12	67.12 ± 4.89
		Second weeks		
T1	26.66 ± 3.2	70.46 ± 4.99	175.28 ± 13.23	67.76 ± 5.34
T2	27.71 ± 2.9	76.61 ± 5.54	169.98 ± 13.00	74.82 ± 4.98
T3	30.07 ± 2.8	80.69 ± 5.69	173.96 ± 14.55	77.9 ± 4.55
T4	34.81 ± 2.0	94.91 ± 6.87	182.44 ± 13.23	79.82 ± 4.87
T5	32.62 ± 3.1	91.34 ± 6.03	177.38 ± 12.89	76.87 ± 5.12
T6	30.44 ± 2.44	85.8 ± 6.69	174.55 ± 12.98	68.91 ± 5.87
T7	29.71 ± 2.9	82.61 ± 6.49	173.87 ± 14.56	73.87 ± 5.14
		Third week		
T1	30.56 ± 3.3	78.26 ± 5.88	189.50 ± 17.87	77.64 ± 5.23
T2	31.61 ± 3.2	82.41 ± 5.90	185.20 ± 15.00	83.7 ± 5.69
T3	32.97 ± 3.1	87.49 ± 6.33	189.18 ± 16.98	84.78 ± 5.45
T4	38.71 ± 2.8	95.71 ± 6.49	198.66 ± 17.00	88.7 ± 5.76
T5	37.52 ± 2.9	94.14 ± 6.54	185.6 ± 15.78	85.75 ± 5.11
T6	36.34 ± 2.6	91.6 ± 6.83	189.77 ± 17.00	77.79 ± 4.87
T7	34.61 ± 2.7	89.41 ± 6.23	183.09 ± 16.98	83.75 ± 4.98
		Fourth week		
T1	36.44 ± 2.6	80.16 ± 5.12	213.38 ± 21.76	88.77 ± 4.89
T2	37.49 ± 2.6	86.31 ± 5.29	209.08 ± 18.99	94.83 ± 5.22
T3	39.85 ± 2.1	91.39 ± 6.74	211.06 ± 17.54	96.91 ± 5.29
T4	43.59 ± 2.2	98.61 ± 7.02	212.54 ± 20.34	98.83 ± 5.87
T5	41.4 ± 2.9	97.04 ± 6.69	211.48 ± 19.00	97.88 ± 5.80
T6	40.22 ± 3.0	95.5 ± 6.34	211.65 ± 18.87	88.92 ± 5.66
T7	38.49 ± 3.2	93.31 ± 5.58	208.97 ± 19.89	86.88 ± 5.42
		Fifth week		
T1	28.67 ± 2.5	73.46 ± 5.67	177.17 ± 17.89	84.22 ± 5.12
T2	30.72 ± 2.9	81.61 ± 5.79	167.87 ± 17.22	90.28 ± 5.23
T3	31.08 ± 2.8	82.69 ± 6.73	174.85 ± 18.91	92.36 ± 5.98
T4	35.82 ± 2.0	89.91 ± 6.09	181.33 ± 16.16	94.28 ± 5.83
T5	34.63 ± 2.0	86.34 ± 6.71	177.27 ± 18.23	91.33 ± 5.12
T6	33.45 ± 2.1	84.8 ± 5.88	175.44 ± 17.77	83.37 ± 5.82
T7	33.72 ± 2.7	83.61 ± 5.74	174.76 ± 18.26	90.33 ± 5.48

T1: 0.9 g sugarcane bagasse + 0.1 g faba bean straw; T2: 0.7 g sugarcane bagasse + 0.3 g faba bean straw; T3: 0.5 g sugarcane bagasse + 0.5 g faba bean straw, T4: 0.3 g sugarcane bagasse + 0.7 g faba bean straw, T5: 0.1 g sugarcane bagasse + 0.9 g faba bean straw, T6: 0.0 g sugarcane bagasse + 1.0 g faba bean straw, T7: 1.0 g sugarcane bagasse + 0.0 g faba bean straw. * Each presented value for FPase, β-glucosidase, CMCase, and soluble P represents the mean (±standard deviation) of three replications of the experiment.

3.5. Concentrations of RP

The data in Table 4 show that soluble P increased with the increase in RP levels, up to 75 mg P_2O_5 from RP under the T4 treatment (0.3 g SCB + 0.7 g FB), and these results were

reflected in cellulase production by *A. japonicus*. The descending order of RP concentration for cellulase production was as follows: 75 mg P_2O_5 > 50 mg P_2O_5 > 25 mg P_2O_5 > 100 mg P_2O_5 (FPase, CMCase, and β-glucosidase); however, as the order was 75 mg P_2O_5 > 50 mg P_2O_5 > 100 mg P_2O_5 > 25 mg P_2O_5 for RP solubilization. The RP plays a critical role during SSF of plant residues. Thus, the content of P in RP should be considered when assessing the quantity of RP. Bio-solubilization of RP is a complex process due to its complex structure and specific particle size.

Table 4. Concentrations of RP cellulase production and P solubilization by *A. japonicus* under different ratios of sugarcane bagasse and faba bean straw residues after 4-week incubation.

Treatment	FPase (Unit g^{-1}) *	β-Glucosidase (Unit g^{-1}) *	CMCase (Unit g^{-1}) *	Soluble P (μg g^{-1}) *
		25 mg P_2O_5		
T1	25.04 ± 2.0	69.12 ± 5.40	176.49 ± 8.20	62.00 ± 3.40
T2	26.69 ± 2.2	74.37 ± 4.78	189.90 ± 9.02	66.77 ± 3.91
T3	26.15 ± 2.4	79.55 ± 5.09	199.47 ± 12.00	67.10 ± 4.18
T4	43.09 ± 1.6	93.17 ± 4.20	221.15 ± 10.05	72.54 ± 4.09
T5	39.10 ± 2.3	89.98 ± 4.40	197.99 ± 10.80	69.22 ± 5.42
T6	36.22 ± 2.9	86.76 ± 5.22	191.26 ± 9.93	62.76 ± 4.87
T7	32.49 ± 1.8	83.97 ± 4.72	182.18 ± 8.52	68.10 ± 4.93
		50 mg P_2O_5		
T1	28.60 ± 2.22	73.41 ± 3.19	198.20 ± 8.03	77.60 ± 5.39
T2	29.11 ± 2.91	77.41 ± 4.24	202.88 ± 8.29	79.44 ± 5.33
T3	36.00 ± 2.63	86.09 ± 4.39	218.90 ± 9.05	83.09 ± 5.15
T4	42.44 ± 2.44	98.71 ± 4.80	249.64 ± 9.13	94.42 ± 5.77
T5	37.43 ± 2.11	90.30 ± 4.83	240.28 ± 9.29	92.80 ± 5.00
T6	33.49 ± 2.15	89.22 ± 4.79	227.05 ± 8.48	88.11 ± 5.08
T7	31.27 ± 2.72	89.49 ± 5.09	210.17 ± 8.96	81.27 ± 4.98
		75 mg P_2O_5		
T1	34.28 ± 3.23	82.26 ± 6.07	199.71 ± 11.43	85.44 ± 6.22
T2	35.04 ± 3.21	85.41 ± 7.20	208.00 ± 11.29	88.51 ± 6.61
T3	39.69 ± 3.11	88.49 ± 7.73	248.10 ± 11.76	101.70 ± 6.35
T4	48.18 ± 2.80	103.71 ± 7.41	267.61 ± 12.33	116.76 ± 6.06
T5	45.34 ± 2.66	96.14 ± 7.54	251.80 ± 11.18	109.85 ± 6.21
T6	42.68 ± 2.82	96.6 ± 7.03	249.77 ± 11.30	103.59 ± 6.57
T7	37.42 ± 2.59	93.41 ± 7.13	231.19 ± 10.18	96.15 ± 6.78
		100 mg P_2O_5		
T1	31.72 ± 2.6	74.67 ± 6.66	190.54 ± 7.23	80.12 ± 6.02
T2	32.13 ± 2.6	81.88 ± 6.12	193.18 ± 7.19	82.60 ± 6.91
T3	35.67 ± 2.1	82.12 ± 6.48	198.08 ± 7.76	91.38 ± 6.65
T4	38.38 ± 2.2	87.75 ± 6.74	217.43 ± 7.18	96.84 ± 6.36
T5	35.74 ± 2.9	83.38 ± 6.86	211.53 ± 7.79	90.72 ± 6.01
T6	31.10 ± 3.0	80.80 ± 6.45	209.62 ± 7.22	88.44 ± 6.27
T7	28.66 ± 3.2	79.05 ± 6.24	201.06 ± 7.05	81.08 ± 6.08

T1: 0.9 g sugarcane bagasse: 0.1 g faba bean straw; T2: 0.7 g sugarcane bagasse + 0.3 g faba bean straw; T3: 0.5 g sugarcane bagasse + 0.5 g faba bean straw, T4: 0.3 g sugarcane bagasse + 0.7 g faba bean straw, T5: 0.1 g sugarcane bagasse + 0.9 g faba bean straw, T6: 0.0 g sugarcane bagasse + 1.0 g faba bean straw, T7: 1.0 g sugarcane bagasse + 0.0 g faba bean straw. * Each presented value for FPase, β-glucosidase, CMCase, and soluble P represents the mean (±standard deviation) of three replications of the experiment.

The earlier experiments showed a potent relationship between the solubilization of insoluble P and the reduction in the pH of the cultivation media. As described by Cerezine et al. [36], the metabolic processes of carbon and nitrogen sources impact total OA synthesis and reduces the pH of the cultivation media, with a consequent impact on the solubilization of insoluble P. In the study by Saber et al. [54] under SSF conditions with two fungal strains—*A. niger* and *Phanerochaete chrysogenum*—as well as rice and wheat

straw, the initial concentration of RP was 75 mg P_2O_5. Moreover, Youssef [59] showed that *Botrytis* sp. MY29, *P. purpurogenum* MY48, *A. niger* MY55, and *A. niger* MY81 were fungi with cellulolytic activities and phosphate solubilization under SSF conditions.

3.6. Inoculum Ratio

As shown in Table 5, the inoculum ratio for cellulase production and soluble P by *A. japonicus* in the T4 treatment (0.3 g SCB: 0.7 g FBS) was found to be 7.5%, which recorded 51.41, 98.31-, and 322.60-units g^{-1} for FPase, β-glucosidase, and CMCase, respectively. Subsequently, when the inoculum size was increased, the cellulase activity decreased, as did soluble P. Inoculum ratios above 7.5% led to a decrease in enzyme synthesis. The decrease in enzyme production was related to the clumping of cells, reducing the sugar and oxygen uptake rates [54,55,60].

Table 5. Effects of different inoculum ratios on cellulase production and P solubilization by *A. japonicus* under different ratios of sugarcane bagasse and faba bean straw residues after the fourth week.

Treatment	FPase (Unit g^{-1}) *	β-Glucosidase (Unit g^{-1}) *	CMCase (Unit g^{-1}) *	Soluble P (μg g^{-1}) *
		2.5% (*v*/*w*)		
T1	33.14 ± 2.5	76.01 ± 4.10	196.21 ± 6.27	69.11 ± 4.00
T2	35.29 ± 3.4	81.19 ± 4.88	202.00 ± 6.02	71.87 ± 4.01
T3	35.78 ± 2.4	79.39 ± 4.11	230.94 ± 6.10	73.75 ± 4.00
T4	43.33 ± 2.4	83.02 ± 4.30	244.41 ± 7.75	81.17 ± 4.19
T5	39.22 ± 3.0	80.91 ± 4.70	222.30 ± 7.07	80.22 ± 4.02
T6	32.10 ± 2.1	71.56 ± 4.42	194.53 ± 7.33	79.16 ± 4.20
T7	29.30 ± 2.0	70.07 ± 4.12	191.80 ± 7.02	72.02 ± 4.69
		5% (*v*/*w*)		
T1	36.56 ± 2.18	72.06 ± 4.09	265.38 ± 7.03	82.44 ± 4.24
T2	37.81 ± 2.23	79.21 ± 4.14	279.08 ± 7.10	87.80 ± 4.78
T3	40.27 ± 2.45	83.19 ± 4.59	290.66 ± 8.35	91.77 ± 4.55
T4	46.01 ± 2.00	95.01 ± 4.47	301.24 ± 8.20	99.02 ± 4.37
T5	45.22 ± 2.11	92.30 ± 4.23	279.08 ± 8.09	96.67 ± 4.02
T6	42.04 ± 2.40	88.81 ± 4.19	274.35 ± 7.18	88.01 ± 4.17
T7	38.11 ± 2.44	82.01 ± 4.09	263.17 ± 7.16	81.97 ± 4.04
		7.5% (*v*/*w*)		
T1	41.36 ± 3.0	90.20 ± 5.08	291.55 ± 8.41	99.69 ± 4.20
T2	44.11 ± 3.1	92.11 ± 5.40	302.21 ± 8.50	101.74 ±4.89
T3	46.57 ± 3.0	97.09 ± 5.03	302.38 ± 8.18	109.38 ± 4.55
T4	51.41 ± 2.3	98.31 ± 5.29	322.60 ± 8.40	114.22 ± 4.46
T5	47.32 ± 2.2	92.12 ± 5.14	308.20 ± 8.28	101.25 ± 4.21
T6	45.30 ± 2.2	90.11 ± 5.33	291.17 ± 8.20	94.09 ± 4.47
T7	43.60 ± 2.1	89.11 ± 5.13	279.29 ± 8.18	89.15 ± 4.28
		10% (*v*/*w*)		
T1	39.46 ± 2.22	78.11 ± 4.72	281.68 ± 7.06	87.43 ± 4.19
T2	42.43 ± 2.43	81.21 ± 4.69	288.48 ± 7.19	92.62 ± 4.02
T3	43.81 ± 2.28	86.09 ± 4.44	291.26 ± 7.24	95.55 ± 4.19
T4	47.50 ± 2.41	90.31 ± 4.12	302.44 ± 7.30	99.21 ± 4.37
T5	40.45 ± 2.53	90.00 ± 4.39	291.38 ± 7.03	95.42 ± 4.20
T6	40.52 ± 2.05	82.54 ± 4.14	281.25 ± 7.80	87.21 ± 4.46
T7	36.49 ± 2.12	75.11 ± 4.08	281.37 ± 7.80	84.11 ± 4.12

T1: 0.9 g sugarcane bagasse: 0.1 g faba bean straw; T2: 0.7 g sugarcane bagasse + 0.3 g faba bean straw; T3: 0.5 g sugarcane bagasse + 0.5 g faba bean straw, T4: 0.3 g sugarcane bagasse + 0.7 g faba bean straw, T5: 0.1 g sugarcane bagasse + 0.9 g faba bean straw, T6: 0.0 g sugarcane bagasse + 1.0 g faba bean straw, T7: 1.0 g sugarcane bagasse + 0.0 g faba bean straw. * Each presented value for FPase, β-glucosidase, CMCase, and soluble P represents the mean (±standard deviation) of three replications of the experiment.

3.7. Production of Organic Acids

The production of organic acids (OAs: mg g^{-1} ± SD) by *A. japonicus* (F15) during SSF of a mixture of sugarcane bagasse and faba bean straw (3:7), in the presence of rock phosphate, was evaluated in the present study. Upon the optimal fermentation process, *A. japonicus* was grown in a mixture of 0.3: 0.7 g SCB: FBS, 75 mg P_2O_5 from RP, and 7.5% (*v*/*w*) fungal inoculum. After 3 weeks of incubation, seven organic acids were detected using HPLC, in the following order of abundance: ascorbic acid (0.531 ± 0.45) > oxalic acid (0.518 ± 0.76) > formic acid (0.287 ± 0.55) > malic acid (0.279 ± 0.67) > succinic acid (0.253 ± 0.53) > lactic acid (0.249 ± 0.57) > citric acid (0.158 ± 0.34 mg g^{-1} waste). The standard curve of organic acids detected by HPLC was found in Figure S1, and the detected organic acids produced by *A. japonicus* were found in Figure S2. The strain F15 could produce OAs/cellulose enzymes, and can be considered a new single-step bio-converter of sugarcane bagasse and faba bean straw residues into OAs. The results indicated that oxalic and ascorbic acids were the most common acids produced as a result of the fungal strain's activities. This study proves the advantage of cheap and abundantly available sugarcane bagasse and faba bean straw residues as a mixture under SSF conditions, using *A. japonicus* MN960315 as a suitable converter of cellulosic biomass into OAs. Our results also show the significance of native fungal isolates—especially *A. japonicus*—and the advantages of the biowastes for the efficient accumulation of various organic acids.

Andersson and Hedlund [61], El-Naggar and El-Hersh [62], and Saber et al. [63] reported that some OAs are produced by fungal strains grown by SSF methods. Moreover, OAs can be detected in hydroxylated fermented products of *A. niger* MY7 No. 81 and *Phanerochaete purpurogenum* MY25 No. 48 after SSF on rice straw in rock phosphate, as reported by several authors. The concentration of salicylic acid was higher than other detected OAs; these findings were reported by Nasr et al. [7] with *A. japonicus*, which produced considerable contents of OAs in the following order in vitro: salicylic > ascorbic > citric > formic > lactic > oxalic and malic acids. Furthermore, this fungus has more efficiency in phosphate solubilization than *Penicillium* spp. The types of OAs affected the solubilization of insoluble phosphates, as well as the kind and status of the practice group in each acid determined to be a prevalent agent that may influence the quantity of emitted P [64].

4. Conclusions

The obtained results indicate the suitability of using cheap and abundantly available sugarcane bagasse and faba bean straw residues as a mixture under solid-state fermentation (SSF) conditions in the presence of *Aspergillus japonicus* MN960315 to convert cellulosic biomass into organic acids (OAs). Of the 15 fungal isolates investigated, *A. japonicus* with a mixture of sugarcane bagasse and faba bean straw (3:7), with 7.5% (*v*/*w*) fungal inoculation, was the best for this fermentation of agricultural waste to produce seven organic acids (i.e., ascorbic acid, oxalic acid, lactic acid, formic acid, succinic acid, citric acid, and malic acid). *A. japonicus* can be used as a promising fungal strain, and represents a valuable approach to reducing environmental contamination. However, further optimization studies should be conducted for exploring the optimal operation conditions to maximize green biosynthesis of OAs and minimize the fermentation period. Consequently, both traditional and modern, novel techniques should be improved and utilized to afford new strains to construct super-producers, together with finding compatible and affordable substrates. Hopefully, fermentation by *Aspergillus*—especially novel industrial filamentous fungal species, which are nontoxic, safe for production, and offer advantages such as higher productivity, higher yields, and lower contamination risk—will be the conventional, all-purpose technique used for the commercial production of natural organic acids in the not-too-distant future.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fermentation8090437/s1. Figure S1: HPLC high performance liquid chromatography spectrum for calibration curve of organic acids. Figure S2: HPLC high performance liquid chromatography spectrum of organic acids produced by *Aspergillus japonicus*.

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References

1. Abou Hussein, S.D.; Sawan, O.M. The utilization of agricultural waste as one of the environmental issues in Egypt (a case study). *J. Appl. Sci. Res.* **2010**, *6*, 1116–1124.
2. Clauser, N.M.; González, G.; Mendieta, C.M.; Kruyeniski, J.; Area, M.C.; Vallejos, M.E. Biomass waste as sustainable raw material for energy and fuels. *Sustainability* **2021**, *13*, 794. [CrossRef]
3. Ganguly, P.; Khan, A.; Das, P.; Bhowal, A. Cellulose from lignocellulose kitchen waste and its application for energy and environment: Bioethanol production and dye removal. *Indian Chem. Eng.* **2021**, *63*, 161–171. [CrossRef]
4. Maki, M.; Leung, K.T.; Qin, W. The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. *Int. J. Biol. Sci.* **2009**, *5*, 500–516. [CrossRef] [PubMed]
5. Dashtban, M.; Schraft, H.; Qin, W. Fungal bioconversion of lignocellulosic residues; opportunities & perspectives. *Int. J. Biol. Sci.* **2009**, *5*, 578–595.
6. Jin, X.; Chen, X.; Shi, C.; Li, M.; Guan, Y.; Yu, C.Y.; Yamada, T.; Sacks, E.J.; Peng, J. Determination of hemicellulose, cellulose and lignin content using visible and near infrared spectroscopy in *Miscanthus sinensis*. *Bioresour. Technol.* **2017**, *241*, 603–609. [CrossRef]
7. Xiao, C.; Tingting, L.; Guang, X.; Ruan, C. Characteristics and mechanisms of biosolubilization of rock phosphate by *Aspergillus japonicus*. *Braz. Arch. Biol. Technol.* **2017**, *60*, e16160541. [CrossRef]
8. Nasr, S.H.; Mousa, A.M.; Marzouk, M.A.; Yasser, M.M. Quantitative and qualitative analysis of organic acids produced by phosphate solubilizing fungi. *Egypt. J. Bot.* **2021**, *61*, 167–176. [CrossRef]
9. Atallah, O.; Yassin, S. *Aspergillus* spp. eliminate *Sclerotinia sclerotiorum* by imbalancing the ambient oxalic acid concentration and parasitizing its sclerotia. *Environ. Microbiol.* **2020**, *22*, 5265–5279. [CrossRef]
10. Li, C.; Huang, C.; Zhao, Y.; Zheng, C.; Su, H.; Zhang, L.; Luo, W.; Zhao, H.; Wang, S.; Huang, L.-J. Effect of choline-based deep eutectic solvent pretreatment on the structure of cellulose and lignin in Bagasse. *Processes* **2021**, *9*, 384. [CrossRef]
11. Irfan, M.; Riaz, M.; Arif, M.S.; Shahzad, S.M.; Saleem, F.; van den Berg, L.; Abbas, F. Estimation and characterization of gaseous pollutant emissions from agricultural crop residue combustion in industrial and household sectors of Pakistan. *Atmos. Environ.* **2014**, *84*, 189–197. [CrossRef]
12. Liska, A.J.; Yang, H.; Milner, M.; Goddard, S.; Blanco-Canqui, H.; Pelton, M.P.; Fang, X.X.; Zhu, H.; Suyker, A.E. Biofuels from crop residue can reduce soil carbon and increase CO_2 emissions. *Nat. Clim. Change* **2014**, *4*, 398–401. [CrossRef]
13. Venkatramanan, V.; Shah, S.; Rai, A.K.; Prasad, R. Nexus between crop residue burning, bioeconomy and sustainable development goals over North-Western India. *Front. Energy Res.* **2021**, *8*, 614212. [CrossRef]
14. Chavan, S.; Yadav, B.; Atmakuri, A.; Tyagi, R.; Wong, J.W.; Drogui, P. Bioconversion of organic wastes into value-added products: A review. *Bioresour. Technol.* **2022**, *344*, 126398. [CrossRef] [PubMed]
15. García-Torreiro, M.; López-Abelairas, M.; Lu-Chau, T.; Lema, J. Fungal pretreatment of agricultural residues for bioethanol production. *Ind. Crops Prod.* **2016**, *89*, 486–492. [CrossRef]
16. Zoghlami, A.; Paës, G. Lignocellulosic biomass: Understanding recalcitrance and predicting hydrolysis. *Front. Chem.* **2019**, *7*, 874. [CrossRef]
17. Pagliaro, P.; Femminò, S.; Penna, C. Redox aspects of myocardial ischemia/reperfusion injury and cardio protection. In *Oxidative Stress in Heart Diseases*; Springer: Singapore, 2019; pp. 289–324.
18. Dadwal, A.; Sharma, S.; Satyanarayana, T. Progress in ameliorating beneficial characteristics of microbial cellulases by genetic engineering approaches for cellulose saccharification. *Front. Microbiol.* **2020**, *11*, 1387. [CrossRef]

19. Zhao, C.; Chu, Y.; Li, Y.; Yang, C.; Chen, Y.; Wang, X.; Liu, B. High-throughput pyrosequencing used for the discovery of a novel cellulase from a thermophilic cellulose-degrading microbial consortium. *Biotechnol. Lett.* **2017**, *39*, 123–131. [CrossRef]
20. Gao, J.; Weng, H.; Zhu, D.; Yuan, M.; Guan, F.; Xi, Y. Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. *Bioresour. Technol.* **2008**, *99*, 7623–7629. [CrossRef]
21. Singh, R.K.; Patil, T.; Verma, A.; Tekade, S.P.; Sawarkar, A.N. Insights into kinetics, reaction mechanism, and thermodynamic analysis of pyrolysis of rice straw from rice bowl of India. *Bioresour. Technol. Rep.* **2021**, *13*, 100639. [CrossRef]
22. Zhou, J.; Wang, Y.-H.; Chu, J.; Zhuang, Y.-P.; Zhang, S.-L.; Yin, P. Identification and purification of the main components of cellulases from a mutant strain of *Trichoderma viride* T 100-14. *Bioresour. Technol.* **2008**, *99*, 6826–6833. [CrossRef]
23. Wang, Y.; Guo, Y.; Cao, W.; Liu, H. Synergistic effects on itaconic acid production in engineered *Aspergillus niger* expressing the two distinct biosynthesis clusters from *Aspergillus terreus* and *Ustilago maydis*. *Microb. Cell Fact.* **2022**, *21*, 158. [CrossRef] [PubMed]
24. Njokweni, S.G.; Steyn, A.; Botes, M.; Viljoen-Bloom, M.; van Zyl, W.H. Potential valorization of organic waste streams to valuable organic acids through microbial conversion: A South African case study. *Catalysts* **2021**, *11*, 964. [CrossRef]
25. Asgher, M.; Asad, M.; Legge, R. Enhanced lignin peroxidase synthesis by *Phanerochaete chrysosporium* in solid state bioprocessing of a lignocellulosic substrate. *World J. Microbiol. Biotechnol.* **2006**, *22*, 449–453. [CrossRef]
26. Kumar, V.; Ahluwalia, V.; Saran, S.; Kumar, J.; Patel, A.K.; Singhania, R.R. Recent developments on solid-state fermentation for production of microbial secondary metabolites: Challenges and solutions. *Bioresour. Technol.* **2021**, *323*, 124566. [CrossRef]
27. Florencio, C.; Cunha, F.M.; Badino, A.C.; Farinas, C.S.; Ximenes, E.; Ladisch, M.R. Secretome analysis of *Trichoderma reesei* and *Aspergillus niger* cultivated by submerged and sequential fermentation processes: Enzyme production for sugarcane bagasse hydrolysis. *Enzyme Microb. Technol.* **2016**, *90*, 53–60. [CrossRef]
28. Tse, T.J.; Wiens, D.J.; Reaney, M.J.T. Production of bioethanol—A review of factors affecting ethanol yield. *Fermentation* **2021**, *7*, 268. [CrossRef]
29. Abdel-Shafy, H.I.; Mansour, M.S. Solid waste issue: Sources, composition, disposal, recycling, and valorization. *Egypt. J. Pet.* **2018**, *27*, 1275–1290. [CrossRef]
30. Barbosa, M.A.G.; Rehn, K.G.; Menezes, M.; Mariano, R.D.L.R. Antagonism of *Trichoderma* species on *Cladosporium herbarum* and their enzimatic characterization. *Braz. J. Microbiol.* **2001**, *32*, 98–104. [CrossRef]
31. Abo-State, M.A.; Shanab, S.M.; Ali, H.E.; Abdullah, M.A. Screening of antimicrobial activity of selected Egyptian cyanobacterial species. *J. Ecol. Heal. Environ.* **2015**, *3*, 7–13.
32. Narasimha, G.; Sridevi, A.; Buddolla, V.; Subhosh, C.M.; Rajasekhar, R.B. Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. *Afr. J. Biotechnol.* **2006**, *5*, 472–476.
33. Ghose, T. Measurement of cellulase activities. *Pure Appl. Chem.* **1987**, *59*, 257–268. [CrossRef]
34. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **1959**, *31*, 426–428. [CrossRef]
35. Nautiyal, C.S. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.* **1999**, *170*, 265–270. [CrossRef]
36. Cerezine, P.C.; Nahas, E.; Banzatto, D.A. Soluble phosphate accumulation by *Aspergillus niger* from fluorapatite. *Appl. Microbiol. Biotechnol.* **1988**, *29*, 501–505. [CrossRef]
37. El-Sawah, M.M.A.; El-Rafey, H.H.; Saber, W.I.A. Reduction of phytic acid content in soy bean by *A. niger* phytase immobilized into gelatin film by cross-linking. *J. Agric. Sci. Mansoura Univ.* **2001**, *26*, 5165–5175.
38. Bonneville, S.; Delpomdor, F.; Préat, A.; Chevalier, C.; Araki, T.; Kazemian, M.; Steele, A.; Schreiber, A.; Wirth, R.; Benning, L.G. Molecular identification of fungi microfossils in a Neoproterozoic shale rock. *Sci. Adv.* **2020**, *6*, eaax7599. [CrossRef]
39. Ramirez, C. *Manual and Atlas of the Penicillia*; Elsevier Biomedical Press: Amsterdam, The Netherlands; New York, NY, USA; Sole Distributors for the USA and Canada, Elsevier/North-Holland: New York, NY, USA, 1982.
40. Raper, K.B.; Fennell, D.I. *The Genus Aspergillus*; Williams and Wilkins: Philadelphia, PA, USA, 1965.
41. Watanabe, T. *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species*; CRC Press: Boca Raton, FL, USA, 2002.
42. Minamisawa, K.; Seki, T.; Onodera, S.; Kubota, M.; Asami, T. Genetic relatedness of *Bradyrhizobium japonicum* field isolates as revealed by repeated sequences and various other characteristics. *Appl. Environ. Microbiol.* **1992**, *58*, 2832–2839. [CrossRef]
43. Xu, C.; Long, M.; Wu, X.; Xu, H.; Chen, Z.; Zhang, F.; Xu, L. Screening and characterization of the high-cellulase-producing strain *Aspergillus glaucus* XC9. *Front. Biol. China* **2006**, *1*, 35–40. [CrossRef]
44. Belal, E.B.; El-Mahrouk, M. Solid-state fermentation of rice straw residues for its use as growing medium in ornamental nurseries. *Acta Astronaut.* **2010**, *67*, 1081–1089. [CrossRef]
45. Chen, Y. Effects of aging-acceleration by electric field on free amino acid of claret. *Liquor Making Sci. Technol.* **2004**, *6*, 80–81.
46. Samson, R.A.; Houbraken, J. *Phylogenetic and Taxonomic Studies on the Genera Penicillium and Talaromyces*; CBS-KNAW Fungal Biodiversity Centre: Utrecht, The Netherlands, 2011.
47. Khalil, M.A.A.; Hashem, H.A. Morphological changes of conidiogenesis in two *Aspergillus* species. *J. Pure Appl. Microbiol.* **2018**, *12*, 2041–2048. [CrossRef]
48. Gams, W.; Christensen, M.; Onions, A.H.; Pitt, J.I.; Samson, R.A. Infrageneric taxa of *Aspergillus*. In *Advances in Penicillium and Aspergillus Systematics*; Springer: Berlin/Heidelberg, Germany, 1986; pp. 55–62.

49. Yang, L.; Lübeck, M.; Lübeck, P. *Aspergillus* as a versatile cell factory for organic acid production. *Fungal Biol. Rev.* **2017**, *31*, 33–49. [CrossRef]
50. Bansal, N.; Soni, R.; Janveja, C.; Soni, S.K. Production of xylanase-cellulase complex by *Bacillus subtilis* NS7 for the biodegradation of agro-waste residues. *Lignocellulose* **2012**, *1*, 196–209.
51. Dhillon, G.S.; Oberoi, H.S.; Kaur, S.; Bansal, S.; Brar, S.K. Value-addition of agricultural wastes for augmented cellulase and xylanase production through solid-state tray fermentation employing mixed-culture of fungi. *Ind. Crops Prod.* **2011**, *34*, 1160–1167. [CrossRef]
52. Liu, J.; Ørskov, E. Cellulase treatment of untreated and steam pre-treated rice straw—Effect on in vitro fermentation characteristics. *Anim. Feed Sci. Technol.* **2000**, *88*, 189–200. [CrossRef]
53. Badhan, A.; Chadha, B.; Kaur, J.; Saini, H.; Bhat, M. Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099. *Bioresour. Technol.* **2007**, *98*, 504–510. [CrossRef]
54. Saber, W.; El-Naggar, N.E.; AbdAl-Aziz, S. Bioconversion of lignocellulosic wastes into organic acids by cellulolytic rock phosphate-solubilizing fungal isolates grown under solid-state fermentation conditions. *Res. J. Microbiol.* **2010**, *5*, 1–20. [CrossRef]
55. El-Nahrawy, S.; Metwally, M.; El-Kodoos, A.; Rizk, Y.; Belal, E.-S.B.; Shabana, S.A.; El-Refai, I.M. Optimization of culture conditions for production of cellulase by *Aspergillus tubingensis* KY615746 using rice straw waste. *Environ. Biodivers. Soil Secur.* **2017**, *1*, 177–189.
56. Narsale, P.V.; Patel, S.R.; Acharya, P. Role of *Aspergillus flavus* on biodegradation of lignocellulosic waste millet straw and optimization parameters for enzyme hydrolysis and ethanol production under solid state fermentation. *Int. J. Curr. Microbiol. Appl. Sci.* **2018**, *7*, 429–445. [CrossRef]
57. Adour, L.; Couriol, C.; Amrane, A.; Prigent, Y. Growth of *Geotrichum candidum* and *Penicillium camembertii* in liquid media in relation with the consumption of carbon and nitrogen sources and the release of ammonia and carbon dioxide. *Enzyme Microb. Technol.* **2002**, *31*, 533–542. [CrossRef]
58. Dutta, D.; Das, M.D. Effect of C/N ratio and microelements on nutrient dynamics and cell morphology in submerged fermentation of *Aspergillus giganteus* MTCC 8408 using Taguchi DOE. *3 Biotech* **2017**, *7*, 34. [CrossRef] [PubMed]
59. Youssef, M. *Organic Acids Associated with the Fungal Degradation of Rice Straw*; MSc. (Microbiology) Botany Department, Faculty of Science, Mansoura University: Mansoura, Egypt, 2016.
60. Ikram-Ul-Haq, U.H.; Shahzadi, K.; Javed, M.; Qadeer, M. Cotton Saccharifying activity of cellulases by *Trichoderma harzianum* UM-11 in shake flask. *J. Bot.* **2005**, *1*, 19–22.
61. Andersson, R.; Hedlund, B. HPLC analysis of organic acids in lactic acid fermented vegetables. *Z. Lebensm. Unters Forsch.* **1983**, *176*, 440–443. [CrossRef] [PubMed]
62. El-Naggar, N.E.-A.; El-Hersh, M.S. Organic acids associated with saccharification of cellulosic wastes during solid-state fermentation. *J. Microbiol.* **2011**, *49*, 58–65. [CrossRef] [PubMed]
63. Saber, W.I.; El-Naggar, N.E.; El-Hersh, M.S.; El-Khateeb, A.Y. An innovative synergism between *Aspergillus oryzae* and *Azotobacter chroococcum* for bioconversion of cellulosic biomass into organic acids under restricted nutritional conditions using multi-response surface optimization. *Biotechnology* **2015**, *14*, 47–57. [CrossRef]
64. Kumari, A.; Kapoor, K.; Kundu, B.; Kumari Mehta, R. Identification of organic acids produced during rice straw decomposition and their role in rock phosphate solubilization. *Plant Soil Environ.* **2008**, *54*, 72. [CrossRef]