Screening of *Aspergillus*, *Bacillus* and *Trichoderma* strains and influence of substrates on auxin and phytases production through solid-state fermentation

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ABSTRACT

Crop inoculation with microorganisms is an agricultural technique that has been used to stimulate plant growth and development by different mechanisms, such as the production of P solubilizing enzymes, the phytases, and indole-3-acetic acid (IAA). The aim of the present study is to produce phytases and IAA *via* solid-state fermentation (SSF) and to correlate biomolecule yield with the characteristics of SSF substrate, such as porosity, water retention, dry mass, electrical conductivity, pH, crude protein, lipids, hemicellulose, cellulose, and lignin for process optimization. Microorganisms belonging to genera *Aspergillus*, *Trichoderma*, and *Bacillus* were cultivated in soybean and wheat bran, in cassava bagasse and in maize and sorghum distiller dried grains with solubles (DDGS). The strains *B. subtilis* (D), *T. atroviride* (IOC 4503), and *Aspergillus niger* (01) produced IAA from tryptophan as shown by LC-MS/MS. All tested microbial genera produced auxins and phytases. The highest indole derivative levels and phytase activity were observed in combinations such as wheat bran/*B. subtilis* (D) and maize DDGS/*T. atroviride*, respectively. There were not strong correlations among substrate property, phytase activity and *Aspergillus* indole compound levels, but there was strong negative correlation between *Trichoderma* indole derivative and lignin levels. The auxin content in genus *Bacillus* had strong negative correlation to lignin and strong positive correlation to pH and hemicellulose; therefore, the production of auxin derivatives by genera *Trichoderma* and *Bacillus* was improved by substrates presenting low lignin content. In addition, substrates with neutral pH and higher hemicellulose content were recommended to *Bacillus*.

1. Introduction

The production of indole-3-acetic acid (IAA) and P-solubilizing enzymes (phytases) by microorganisms are among mechanisms widely used to stimulate plant growth in agriculture (Acuña and Jorquera, 2011).

IAA is associated with many physiological processes in plants, such as apical dominance, tropisms, shoot elongation, cambial cell division induction and root initiation (Zhao, 2010). Several classes of microorganisms have the ability to produce IAA, including plant pathogens, symbionts, rhizobacteria and microorganisms rarely associated with plants (Spaepen et al., 2007). Microbial associations with both root system and rhizosphere can play a key role in auxin availability to plants, as well as in increasing plant resistance to diseases and in contributing to plants' nutrient and water absorption (Almeida et al., 2017).

Phytases increase phosphorus (P) availability in the soil and improve plant development. Organic P (Po) prevails (80%) in the soil, and half of it is found in its phytate form (Na-IHP), which is marginally used by plants as phosphorus source (Hayes et al., 2000; Richardson et al., 2000). Plants or microbial phytases can hydrolyze phytate enzymes through the production of orthophosphates, which are the sources of P absorption available to plants (George et al., 2007).

Microbial biomolecules, such as auxins and phytases, can be produced through fermentation, because microorganisms metabolize nutrients, synthesize secondary metabolites and complete other metabolic activities when they are subjected to aerobic and anaerobic conditions

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during the fermentation process. There are two fermentation types, namely: submerged (SmF) and solid-state fermentations (SSF). SmF is the most commonly used fermentation technique given its uniformity and easy temperature control; however, it has some disadvantages, such as high energy consumption and strong polluting profile, fact that impairs process sustainability (Chen, 2013). On the other hand, SSF requires no, or low amounts of, water in the substrates, whose moisture levels must be enough to support microorganisms' growth and metabolic activity (Thomas et al., 2013). Overall, SSF substrates are agro-industrial wastes and by-products that help solving surplus disposal issues (Singh Nee Nigam and Pandey, 2009).

The SSF process can be regulated by changes in the macroscopic characteristics of the substrates, such as dry mass and porosity, dynamical features during the fermentation process and microscopic characteristics mainly including microbial growth, substrate surface adhesion and substrate decomposition by microorganisms (Chen, 2013). Substrates are carbon and energy sources for microorganisms, since their common structure is mainly composed of cellulose, starch, pectin and lignocellulose (Singhania et al., 2016). However, the influence of each component on the industrial production of auxin and phytases through SSF remains unknown.

The aim of the present research was to investigate whether *Aspergillus* spp., *Bacillus* spp. and *Trichoderma* spp. strains can produce auxin and phytases through SSF in different substrates, as well as to optimize the auxin and phytases production process by better understanding the influence of the physical and chemical characteristics of the substrates on the production of these biomolecules.

2. Materials and methods

2.1. Inoculum preparation

Strains of *Aspergillus ustus* (Bainer.) Thom & Church (IOC 4410), *Aspergillus niger* van Tieghem (INCQS 40015), *Trichoderma atroviride* (IOC 4503), *Trichoderma koningii* (INCQS 40331), *Trichoderma harzianum* Rifai (IOC 3844), *Bacillus subtilis* (CCGB 0030), *Bacillus megaterium* (CCGB 0146) and *Bacillus amyloliquefaciens* (CCGB 0145) were provided by Oswaldo Cruz Foundation (FIOCRUZ). The fungal species *Aspergillus niger* 01 was isolated from a thermally heated soil; next, it was morphologically identified and the sample was deposited in the Brazilian Collection of Environmental and Industrial Microorganisms under registration n. CBMAI 2084. *Bacillus subtilis* strains B, C, D, E, F and 27 were provided by the bacteria collection of the Food Biochemistry Laboratory, Campinas State University (UNICAMP), Brazil. All microorganism species were registered under n. A3B348F in the platform of the National Genetic Heritage Management System (SISGEN), as recommended by the Brazilian Biodiversity Law (n. 13.123/15).

Fungi were grown in potato dextrose agar slants for 96 h at 30 °C. Bacteria were grown in tryptic soy agar slants for 24 h at 33 °C. Slants were stored at 4 °C after growth, for further use. The SSF inoculum was prepared through the suspension of the slant content in 6 mL of sterile distilled water. The suspension was adjusted to 10^7 spores mL^{-1} - 2 mL of it was used to inoculate the medium.

2.2. Solid-state fermentation (SSF)

Cassava bagasse (donated by the starch producer "Flor de Lotus", located in Cândido Mota County, São Paulo State, Brazil), soybean bran, wheat bran, sorghum and maize distiller dried grains with solubles (DDGS) (provided by the feed mill of the School of Veterinary Medicine and Animal Science of UNESP, located in Botucatu County, São Paulo State, Brazil) were the substrates used to test auxin and phytase production through SSF. Tryptophan, in its powder form, (1%, w/w) was added to the substrates to induce auxin synthesis.

The medium used in the SSF process was composed of 50% substrate and 50% distilled water (w/w). The medium (10 g) was inoculated with 10^7 spores mL^{-1} suspension in 250 mL Erlenmeyer flasks and incubated at 30 °C for 120 h (Novelli et al., 2016). Mycelia were removed from the slant by adding 50 mL of distilled water to it and by filtering the suspension through two layers of cotton gauze. The filtrate was used to measure indole derivatives and to conduct the phytase assay.

2.3. Phytase activity

Phytase activity was determined in reaction medium composed of 1 mL of 5 mM p-nitrophenyl phosphate (Sigma-Aldrich®, St Louis, USA), 0.5 mL of 0.8 M acetate buffer and 10–100 μL of crude enzyme extract at pH 5.0. The mixture was incubated for 10 min at 37 °C. The reaction was stopped through the addition of 2 mL of 0.1 M sodium hydroxide. Absorbance readings were performed at 410 nm. The control sample was determined through crude enzyme extract denaturation, which was based on extract boiling for 5 min. Results were expressed as the standard curve of *p*-nitrophenol (Stöckmann et al., 2003). Total proteins in microbial extracts were quantified in order to determine the specific activity of phytases. The reaction medium was composed of 100 μL of crude microbial extracts, 900 μL of sodium sulfite 24% (w/v) and 4.0 mL of Biuret reagent. The mixture was incubated for 30 min at room temperature. Absorbance readings were performed at 535 nm. Results were calculated through standard curve by using from 0 to 9 mg mL^{-1} of casein (Gornall et al., 1949). Phytase activity was expressed as U mg protein^{-1}.

2.4. Spectrophotometric quantification of indole derivatives

Auxin quantification was carried out through the reaction of 1 mL of SSF extract with 4 mL of Salkowski reagent (2% FeCl$_3$ in 35% perchloric acid). Absorbance was read after rest at room temperature for 30 min, at 535 nm; next, the absorbance results were compared to the standard IAA curve. Blank was composed of 1 mL of substrate extract added with tryptophan (1%, w/w) and 4 mL of Salkowski reagent. Results were expressed as μg of indole derivatives mL^{-1} (Bric et al., 1991; Sarwar et al., 1992). The test based on substrate supplementation with IAA at doses 40, 80, 120 and 160 μg mL^{-1} was carried out to make sure that IAA would recover from the substrate.

2.5. Indole-3-acetic acid (IAA) determination through LC-MS/MS

A. niger (CBMAI, 01) extracts cultivated in wheat bran, *B. subtilis* D grown in wheat bran and *T. atroviride* (IOC 4503) cultivated in soybean bran were prepared to be injected in LC-MS/MS. The mixtures were centrifuged at 6,000 rpm for 10 min and filtered through 0.22 μm membrane. The LC-MS/MS system was used to quantify IAA, it was composed of High Efficiency Liquid Chromatograph (HPLC), model Proeminence UFLC (Shimadzu®, Kyoto, Japan) equipped with two pumps LC-20AD, self-injector SIL-20AC, degasser DGU-20A5, controller system CBM-20A and oven CTO-20AC - HPLC was coupled to a the mass spectrometer 3200 Q TRAP (Applied Biosystems®, Foster City, USA).

Synergy Fusion RP 100 Å chromatographic column (Phenomenex) (2.5 μm) was used in the experiment; it used 1% acetic acid diluted in milli-q water (v/v) (phase A) and 1% acetic acid diluted in methanol (v/v) (phase B) as mobile phase at flow rate 0.65 mL min^{-1}. The following gradient was adopted for the trial: 0–2 min, 45% phase B; 2–8 min, 95% phase B; and 8–10 min, 45% phase B. Total run time was 10 min; compound retention time in the chromatographic column was 2.44 min. LC-MS/MS was operated in negative ion mode electrospray ionization (ESI).

2.6. Determination of substrate's physical and chemical properties

Chemical properties crude protein, lipids, hemicellulose, cellulose, lignin, pH and electrical conductivity, (AOAC, 2000), as well as

physical properties macroporosity, microporosity, total porosity and water retention (Guerrini and Trigueiro, 2004) of the SSF were determined.

2.7. Statistical analysis

Experimental results were expressed as the mean values of three biological and three technical replicates (mean ± SD). Significant differences between samples were evaluated through analysis of variance (ANOVA), which was followed by Scott-Knott test ($p \leq 0.05$). Biomolecule data were linearly correlated to the physical and chemical properties of different substrates through Pearson's correlation ($p \leq 0.01$), which is the coefficient between two variables (from +1 to −1), wherein 1 is the total positive linear correlation, 0 means lack of linear correlation and −1 is the total negative linear correlation.

3. Results and discussion

3.1. Indole-3-acetic acid and indole derivative production

To the best of our knowledge, the present study is pioneer in confirming IAA production through solid-state fermentation based on LC-MS/MS. The analysis showed that *B. subtilis* D strains cultivated in wheat bran, *T. atroviride* (IOC 4503) grown in soybean bran and *A. niger* (CBMAI, 01) cultivated in wheat bran can produce IAA, as well as that tryptophan increased the amount of IAA by approximately 10-fold (Fig. 1). The spectrophotometric method, whose overall indole derivative level could be estimated was used, since the study on auxin derivative production by microorganisms associated with fermentation substrates involved high sampling. Based on the outcomes, exogenously added IAA could be extracted from soybean bran, cassava bagasse, wheat bran, sorghum and maize DDGS substrates. Soybean and wheat bran were the best substrates for IAA recovery. Overall, just cassava bagasse followed a linear model for IAA concentration increase (Fig. 2).

Based on the results, most analyzed strains were able to produce indole derivatives in different substrates. In total, 26 combinations recorded production rates above $50\,\mu g\,mL^{-1}$. In addition, there were differences in the production rates of indole derivatives depending on the species and on the substrate.

Results on the production of indole derivatives (ca $158\,\mu g\,mL^{-1}$) showed the potential of *Bacillus subtilis* D strain grown in wheat bran, since its result was higher than the ones recorded for other substrate/strain combinations (Table 1). The high indole derivative production by *Bacillus subtilis* D was followed by that of *B. amyloliquefaciens* (CCGB 0145) strain cultivated in soybean bran (ca $141\,\mu g\,mL^{-1}$). *B. subtilis* (CCGB 0030) strain cultivated in soybean bran, *B. subtilis* E27 grown in wheat bran and *B. megaterium* (CCGB 0146) cultivated in soybean and
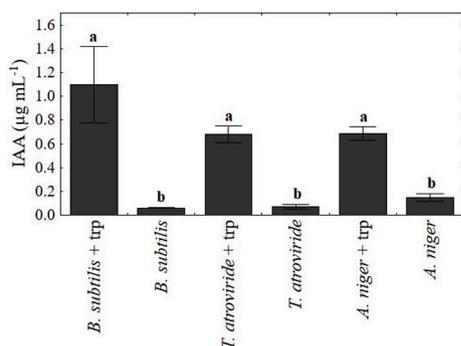
in wheat bran presented the same ability to produce indole derivatives (ca $110\,\mu g\,mL^{-1}$).

The potential of *Bacillus* to produce auxins has been described in several studies. Endophytic *Bacillus* spp. strains isolated from medicinal plants in Bangladesh and cultivated in Jensen's broth with $2\,mg\,mL^{-1}$ L-tryptophan produced from 6 to $63\,\mu g\,mL^{-1}$ IAA. Two potential isolates were identified as *B. subtilis* BRtL-2 and *B. amyloliquefaciens* BDR-2 by 16S rRNA gene sequencing (Ansary et al., 2018).

Bacillus spp. strains grown in broth at different L-tryptophan concentrations (150 and $300\,\mu g\,mL^{-1}$) generated different amounts of IAA. *Bacillus* sp. BHUJP-H2 produced $26\,\mu g\,mL^{-1}$ IAA, regardless of the tryptophan concentration. *B. subtilis* BHUJP-H1 and *B. licheniformis* BHUJP-H3 produced 15 and $32\,\mu g\,mL^{-1}$ IAA, respectively, due to lower tryptophan addition, as well as 25 and $35\,\mu g\,mL^{-1}$ IAA, due to higher tryptophan addition. The following strain combinations, *Bacillus subtilis* BHUJP-H1, *B. subtilis* BHUJP-H1C + *B. licheniformis* BHUJP-H3. *B. subtilis* BHUJP-H1C + *Bacillus* sp. BHUJP-H2C + *B. licheniformis* BHUJP-H3 were described as the best treatments to enhance mung bean (*Vigna radiata*) growth in comparison to the control and to the other combinations (Verma et al., 2018).

The endophytic strain *B. subtilis* 10-4 cultivated in Luria-Bertani broth - supplemented with $1\,mg\,mL\,L^{-1}$ tryptophan - produced $5.8\,\mu g\,mL^{-1}$ IAA. Plant growth and biomass recorded significant increase under non-saline and saline (2% NaCl) conditions, when it was inoculated in wheat species *Triticum aestivum* L. (Lastochkina et al., 2017).

Trichoderma combinations of *T. koningii* (INCQS 40331) in sorghum DDGS and *T. atroviride* (IOC 4503) combinations in soybean bran also showed higher auxin derivative production: 108 and $103\,\mu g\,mL^{-1}$, respectively (Table 2). Based on the literature, the production of metabolites with the potential to promote plant growth was verified in 101 *Trichoderma* genotypes from Colombia - 60% of them had the ability to produce indole-3-acetic acid or auxin analogues (Hoyos-Carvajal et al., 2009). In total, 70 *Trichoderma* spp. strains isolated from the rhizosphere of Indian soils were inoculated in czapek-dox broth and produced from 0.7 to $1.6\,\mu g\,mL^{-1}$ of indole derivatives in tryptophan-free medium, as well as from 5.5 to $36.4\,\mu g\,mL^{-1}$ in tryptophan medium ($200\,\mu g\,mL^{-1}$). One of these isolates (TRC3) was the most effective to increase *Zea mays* shoot, root length, leaf area and total biomass, as well as stem and leaf fresh weight in plants subjected to stress caused by salinity conditions (Kumar et al., 2017).

The production of indole derivatives by *B. subtilis* D cultivated in wheat bran in the present study was 3–26-fold higher than results published in the literature (Ansary et al., 2018; Lastochkina et al., 2017; Verma et al., 2018), whereas the indole derivatives production by *T. koningnii* cultivated in sorghum DDGS and by *T. atroviride* (IOC 4503) grown in soybean bran was 3–18-fold higher than outcomes in the literature (Hoyos-Carvajal et al., 2009; Kumar et al., 2017). Indole derivative production by *Bacillus* and *Trichoderma* subjected to SSF was higher than that of those subjected to SmF. Furthermore, solid-state fermentation required low-cost culture media (agro-industrial waste), whereas SmF required more complex and expensive culture media. The product from SSF fermentation can be directly applied to the soil; thus, this is an advantageous technique for agricultural purposes.

3.2. Phytase production

Based on results recorded for phytase production, only few combinations (microorganism-substrate) could produce high–activity extracts - only 14 combinations recorded activity higher than 80 U mg protein^{-1}. *A. niger* (Table 3) and *T. atroviride* (IOC 4503) (Table 2) recorded the highest activity rates.

T. atroviride (IOC 4503) cultivated in maize DDGS, which reached ca 2000 U mg protein^{-1}; and *A. niger* (CBMAI, 01) grown in wheat bran, with 1500 U mg protein^{-1}, were the combinations leading to the highest enzymatic activity rates of phytase production. Different from

Fig. 1. LC-MS/MS analysis of indole-3-acetic acid (IAA) ($\mu g\,mL^{-1}$) production in the extracts of *B. subtilis* D in wheat bran, *T. atroviride* in soybean bran and *A. niger* 01 in wheat bran, with and without tryptophan (trp) (1% w/w) addition. Means of three replicates followed by the same letter do not differ statistically by Scott Knott test ($p \leq 0.05$).
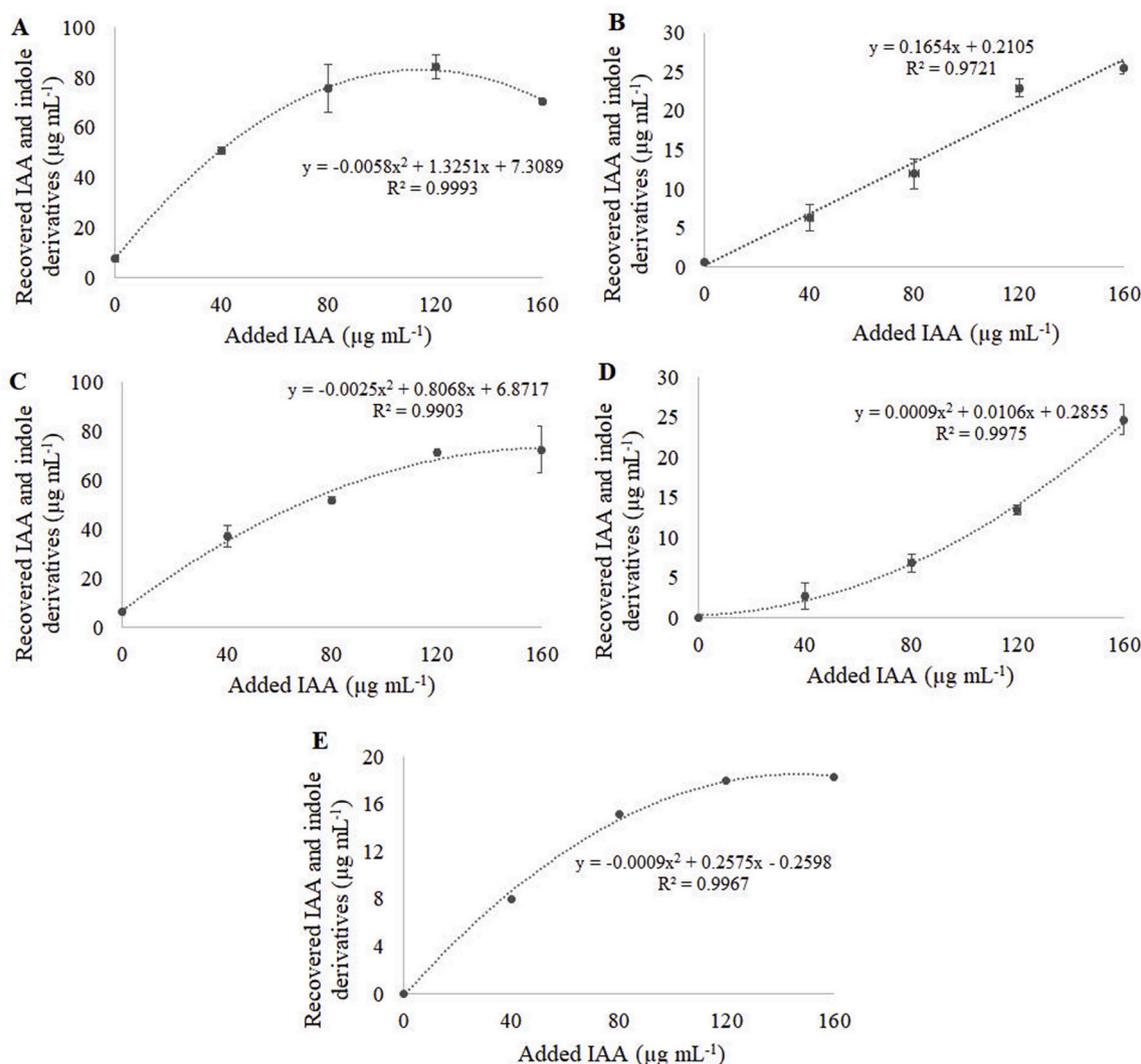
Fig. 2. Extraction of indole-3-acetic acid (IAA) (0, 40, 80, 120 and 160 μg mL^{-1}) and derivatives from the supports soybean bran (A), cassava bagasse (B), wheat bran (C), sorghum DDGS (D) and maize DDGS (E).
Means of three replicates $\pm$ SD.

the production of auxin derivatives, *Bacillus* strains showed the lowest phytase activity, from 0.3 to 2.0 U mg protein $^{-1}$ (Table 1).

Phytase production by *A. niger* and *Trichoderma* species has been reported in the literature, and results in these publications confirm the present ones; therefore, microorganisms have been applied to plants as a way to stimulate plant growth. Based on the literature, phytase production by *A. niger* through SSF in wheat bran substrate is expected to reach 38 U g^{-1} substrate after 5-incubation days. It was possible achieving Phytase activity increase to 50 U g^{-1} in wheat bran, rice bran and groundnut cake at ratio 2:1:1 (w/w) (Shivanna and Venkateswaran, 2014). Phytase genes of *A. niger* were incorporated to plants through genetic transformation. The modified plants acquired the ability to release fungal extracellular phytase and to absorb P from phytate (Richardson et al., 2005).

Trichoderma asperellum Q1 grown in liquid medium produced up to 0.17 U mL^{-1} phytase, it was able to degrade high amounts of tricalcium phosphate and dibasic calcium phosphate (Zhao and Zhang, 2015). Ten (10) *Trichoderma* strains of *A. marina* rizosphere produced from 160 to 350 U mL^{-1} phytase and solubilized from 1 to 29% Ca$_3$ (PO$_4$)$_2$ *in vitro*. This outcome demonstrates the potential of this fungal genus to increase soil fertility and to improve plant growth in mangrove zones (Saravanakumar et al., 2013).

The low phytase activity of *Bacillus* species was also corroborated by

previous studies; *Bacillus* sp. KHU-10 subjected to optimum conditions produced 0.2 U mL^{-1} of phytase. Despite their low production, these enzymes are active at neutral pH, they show high thermal stability and substrate specificity for the calcium-phytate complex, which are characteristics that favor commercial and environmental application (Fu et al., 2008).

3.3. Influence of substrates' properties on the production of auxins and phytases

There was large variation in the production of indole derivatives and phytases by the same microorganism cultivated in different substrates. *Bacillus subtilis* D did not produce auxins in cassava bagasse, whereas this strain reached 158 μg mL^{-1} in wheat bran. Phytase production by *T. atroviride* (IOC 4503) ranged from 28 U mg^{-1} of protein in cassava bagasse to 2080 U mg^{-1} of protein in maize DDGS. The lowest yield of both biomolecules in all microorganisms was observed in cassava bagasse, which is composed of carbohydrates, mainly of starch. Thus, microorganisms that use starch as substrate to achieve growth and other metabolic activities have been selected for the bioconversion process carried out in this substrate (Pandey et al., 2000). Based on the present results, other substrate components exerted great influence on bioconversion due to the low production of auxin and

Table 1

Production of phytase (U mg protein^{-1}) and indole derivatives (μg indole-3-acetic acid equivalents mL^{-1}) by *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus amyloliquefaciens* cultivated through solid-state fermentation in soybean bran (SB), wheat bran (WB) and cassava bagasse (CB).

Microorganisms	Fermentation substrates	Phytase activity (U mg protein^{-1})	Indole derivatives (μg mL^{-1})
B. subtilis 0030	SB	0.48 ± 0.10 g	111.7 ± 22.8 b
B. subtilis 0030	WB	0.67 ± 0.09 g	86.7 ± 9.5 c
B. subtilis 0030	CB	0.58 ± 0.16 g	7.9 ± 4.0 f
B. subtilis B	SB	1.45 ± 0.18 g	75.3 ± 13.8 d
B. subtilis B	WB	1.01 ± 0.16 g	45.9 ± 6.6 e
B. subtilis B	CB	0.52 ± 0.08 g	6.2 ± 4.6 f
B. subtilis C	SB	1.56 ± 0.18 g	57.3 ± 4.5 d
B. subtilis C	WB	1.11 ± 0.03 g	85.2 ± 12.6 c
B. subtilis C	CB	0.88 ± 0.17 g	12.6 ± 3.2 f
B. subtilis D	SB	2.03 ± 0.05 g	78.0 ± 1.9 d
B. subtilis D	WB	1.00 ± 0.20 g	158.4 ± 16.8 a
B. subtilis D	CB	0.66 ± 0.06 g	–
B. subtilis E	SB	0.61 ± 0.09 g	78.6 ± 2.8 d
B. subtilis E	WB	0.46 ± 0.07 g	93.8 ± 16.6 c
B. subtilis E	CB	0.43 ± 0.03 g	3.2 ± 2.9 f
B. subtilis F	SB	0.76 ± 0.05 g	33.3 ± 3.2 e
B. subtilis F	WB	0.56 ± 0.08 g	89.3 ± 15.4 c
B. subtilis F	CB	1.64 ± 0.29 g	5.1 ± 2.5 f
B. subtilis 27	SB	0.90 ± 0.08 g	74.0 ± 5.6 d
B. subtilis 27	WB	2.00 ± 0.54 g	110.5 ± 13.4 b
B. subtilis 27	CB	1.00 ± 0.14 g	7.7 ± 3.7 f
B. amyloliquefaciens	SB	0.42 ± 0.09 g	140.8 ± 6.7 a
B. amyloliquefaciens	WB	0.33 ± 0.04 g	90.9 ± 2.2 c
B. amyloliquefaciens	CB	0.68 ± 0.05 g	13.1 ± 2.0 f
B. megaterium	SB	0.67 ± 0.06 g	116.5 ± 10.3 b
B. megaterium	WB	1.06 ± 0.25 g	118.4 ± 6.7 b
B. megaterium	CB	0.80 ± 0.05 g	13.3 ± 2.0 f

Means followed by the same letter did not statistically differ in the Scott Knott test (p ≤ 0.05).

Table 2

Production of phytase (U mg protein^{-1}) and indole derivatives (μg indole-3-acetic acid equivalents mL^{-1}) by *Trichoderma harzianum*, *Trcihoderma koningii* and *Trichoderma atroviride* cultivated through solid-state fermentation in soybean bran (SB), wheat bran (WB), cassava bagasse (CB), maize (DM) and sorgum (DS) distiller dried grains with solubles.

Microorganisms	Fermentation substrates	Phytase activity (U mg protein^{-1})	Indole derivatives (μg mL^{-1})
T. koningii	SB	5.78 ± 0.53 g	70.5 ± 33.8 d
T. koningii	WB	50.8 ± 18.7 g	70.5 ± 9.7 d
T. koningii	CB	11.7 ± 0.13 g	23.6 ± 1.4 e
T. koningii	DS	60.4 ± 12.3 g	108.1 ± 5.5 b
T. koningii	DM	37.4 ± 5.1 g	65.8 ± 8.2 d
T. harzianum	WB	19.7 ± 2.5 g	97.3 ± 0.24 c
T. harzianum	CB	23.3 ± 2.0 g	19.9 ± 1.3 f
T. harzianum	DS	109.2 ± 17.2 g	95.3 ± 42.4 c
T. harzianum	DM	937.9 ± 65.5 c	33.0 ± 2.3 e
T. atroviride	SB	541.3 ± 65.3 e	103.3 ± 7.1 b
T. atroviride	WB	784.3 ± 305.5 d	60.8 ± 3.3 d
T. atroviride	CB	28.6 ± 2.4 g	1.1 ± 0.47 f
T. atroviride	DS	1013.2 ± 132.7 c	24.3 ± 2.7 e
T. atroviride	DM	2084.8 ± 405.5 a	30.5 ± 2.9 e

Means followed by the same letter did not statistically differ in the Scott Knott test (p ≤ 0.05).

phytase in cassava bagasse. These differences were the reasons motivating the present study, which investigated the properties of substrates used in SSF processes and correlated such properties to the levels recorded for these biomolecules.

Some species did not grow in the tested substrates, among them, one finds *A. ustus* (IOC 4410) cultivated in soybean bran, in cassava bagasse and in maize DDGS; *T. harzianum* (IOC 3844) cultivated in soybean bran and all *Bacillus* strains grown in maize and sorghum DDGS. Results recorded for *Bacillus* species can be justified by the low pH observed in

Table 3

Production of phytase (U mg protein^{-1}) and indole derivatives (μg indole-3-acetic acid equivalents mL^{-1}) by *Aspergillus niger* and *Aspergillus ustus* cultivated through solid-state fermentation in soybean bran (SB), wheat bran (WB), cassava bagasse (CB), maize (DM) and sorgum (DS) distiller dried grains with solubles.

Microorganisms	Fermentation substrates	Phytase activity (U mg protein^{-1})	Indole derivatives (μg mL^{-1})
A. niger 01	SB	358.5 ± 40.9 f	37.7 ± 28.3 e
A. niger 01	WB	1536.4 ± 169.0 b	19.2 ± 14.8 f
A. niger 01	CB	84.3 ± 18.3 g	11.9 ± 1.9 f
A. niger 01	DS	332.6 ± 61.3 f	32.5 ± 4.0 e
A. niger 01	DM	541.4 ± 142.7 e	19.9 ± 1.0 f
A. niger 15	SB	267.0 ± 31.0 f	26.9 ± 0.70 e
A. niger 40015	WB	332.9 ± 50.8 f	7.8 ± 0.22 f
A. niger 40015	CB	8.55 ± 0.45 g	17.2 ± 1.2 f
A. niger 40015	DS	43.5 ± 8.6 g	45.1 ± 2.6 e
A. niger 40015	DM	333.1 ± 30.3 f	20.5 ± 1.5 f
A. ustus	WB	1.3 ± 0.18 g	81.3 ± 7.4 c
A. ustus	DS	9.3 ± 1.2 g	61.2 ± 11.5 d

Means followed by the same letter did not statistically differ in the Scott Knott test (p ≤ 0.05).

Table 4

Physical and chemical properties: macroporosity (MaP) (%, w/w), microporosity (MiP) (%, w/w), total porosity (TP) (%, w/w), water retention (WR) (mL cm^{-3}), electric conductivity (EC) (mS cm^{-3}), pH, crude protein (CP) (%, w/w), lipids (%, w/w), hemicellulose (%, w/w), cellulose (%, w/w) and lignin (%, w/w) of soybean bran (SB), wheat bran (WB), cassava bagasse (CB), maize (DM) and sorgum (DS) distiller dried grains with solubles.

Physical and chemical properties	Substrates				
	SB	WB	CB	DS	DM
Macroporosity (%)	13.86	29.84	33.10	36.95	45.23
Microporosity (%)	59.09	47.43	40.28	38.90	36.90
Total porosity (%)	72.95	77.28	73.38	75.84	82.13
Water retention (mL cm^{-3})	0.56	0.45	0.38	0.37	0.35
Electric conductivity (mS cm^{-3})	3.10	0.78	0.69	0.60	0.70
pH	6.48	5.91	4.33	4.20	4.04
Crude protein (%)	46.65	17.00	3.32	36.92	30.16
Lipids (%)	8.76	6.35	8.91	9.58	8.09
Hemicellulose (%)	15.84	28.88	9.96	36.82	43.87
Cellulose (%)	5.56	10.77	10.29	15.22	15.28
Lignin (%)	2.43	4.77	27.00	14.69	13.60

maize and sorghum DDGS, respectively, which were determined as ca 4 (Table 4). Bacterial and fungal growth rates were inversely proportional to pH. Bacteria grew fast in pH close to 7, although fungi can grow in pH between 4.5 and 8.3; however, there was total growth inhibition of all microorganisms under lower pH conditions (Rousk et al., 2009).

The microorganisms belonging to each genus was subjected to correlation analysis to allow the investigation of differences in microbial metabolism (Table 5). There were not significant correlations between indole derivative and phytase production in genus *Aspergillus*. There was not significant correlation in phytase production by *Bacillus*, but its production by *Trichoderma* showed moderate positive correlation to total porosity and hemicellulose content. There was strong negative correlation between *Trichoderma* and lignin (−0.688) in the production of indole derivatives, and moderate positive correlation to microporosity (0.575), water retention (0.571) and crude protein (0.529) in *Bacillus*, as well as moderate negative correlation to lipids (−0.538), strong positive correlation to pH (0.772) and hemicellulose (0.683) and strong negative correlation to lignin (−0.819) (Table 5).

A similar correlation coefficient was recorded for microporosity and water retention in both microbial genera, due to molecular adhesion caused by adsorption, since micropores retain more water at higher voltages than macropores, and this process reduces water availability

Table 5

Pearson's coefficients calculated for auxin derivative and phytase production by *Bacillus*, *Trichoderma* and *Aspergillus*; physical and chemical substrate properties: macroporosity (MaP) (%, w/w), microporosity (MiP) (%, w/w), total porosity (TP) (%, w/w), water retention (WR) (mL cm^{-3}), electric conductivity (EC) (mS cm^{-3}), pH, crude protein (CP) (%, w/w), lipids (%, w/w), hemicellulose (%, w/w), cellulose (%, w/w) and lignin (%, w/w).

| Substrate properties | *Bacillus* | | Microorganisms | | *Aspergillus* | |
| | | | *Trichoderma* | | | |
	Indole derivatives (µg mL^{-1})	Phytase (U mg ptn^{-1})	Indole derivatives (µg mL^{-1})	Phytase (U mg ptn^{-1})	Indole derivatives (µg mL^{-1})	Phytase (U mg ptn^{-1})
MaP (%)	−0.422	−0.142	−0.359	0.358	−0.074	−0.044
MiP (%)	0.575*	0.155	0.450	−0.224	0.069	0.166
TP (%)	0.443	0.003	−0.029	0.528*	−0.060	0.280
WR (mL cm^{-3})	0.571*	0.155	0.448	−0.222	0.067	0.165
EC (mS cm^{-3})	0.330	0.132	0.352	−0.093	−0.001	0.023
pH	0.772*	0.158	0.486	−0.211	0.098	0.293
CP (%)	0.529*	0.152	0.573	0.252	0.296	−0.200
LP (%)	−0.538*	−0.025	−0.149	−0.058	0.049	−0.489
H (%)	0.683*	0.065	0.238	0.493*	0.215	0.164
C (%)	−0.239	−0.121	−0.120	0.355	0.115	−0.069
L (%)	−0.819*	−0.151	−0.688*	−0.130	−0.263	−0.391

*$p \leq 0.01$.

(Silva et al., 2012). The strong positive correlation between pH and the production of indole derivatives by *Bacillus* can be explained by better bacteria adaptability and by the consequent higher production of metabolites in environments at pH close to neutral (Rousk et al., 2009).

The positive correlation between hemicellulose and auxin derivatives, as well as the negative correlation between lignin and auxin derivatives, can be explained by the complexity and energetic conversions of different polymers. Hemicelluloses are mainly composed of xylan and their degradation require the activity of several hydrolytic enzymes, which result in monosaccharides and acetic acid (Pérez et al., 2002). Extracellular, oxidative and nonspecific enzymes are needed for lignin depolymerization given their structural complexity, high molecular weight and insolubility - all these factors hamper lignin degradation (Kirk and Farrell, 1987). The energetic conversion of hemicellulose was simpler than that of lignin, and this outcome may have favored indole derivative production by *Bacillus*.

Several microorganisms are capable of degrading cellulose, hemicellulose and lignin; therefore, processes based on enzymes produced by microorganisms have been developed in order to exploit their biotechnological potential, since polymers can be used as substrate for microorganism growth. The degradation of these enzymes can release molecules of industrial interest (Pérez et al., 2002).

The herein assessed microorganisms were capable of producing enzymes that degrade lignocellulose. *Bacillus* sp., *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus fumigatus Trichoderma reesei* and *Trichoderma viride* were cellulases producers: β-glucosidase, cellobiohydrolase or exoglucanase and glycosyltransferase. *Bacillus* sp., *Aspergillus niger*, *Aspergillus nidulans* and *Aspergillus fumigatus* were hemicellulases producers: xylanase, mannase, endoglucanase, β-xylodase, α-galactosidase, acetyl esterase and β-glucosidase. *Bacillus subtilis*, *Trichoderma reesei* and *Trichoderma longibrachiatum* were lignase producers: laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase and cellobiose dehydrogenase (Sharma et al., 2017). Based on the present findings, and on results in the literature, further research is recommended in order to test whether the activity of lignocellulases can be a new study field for the biotechnological application of these strains.

4. Conclusion

Results in the present study confirmed the IAA production through solid-state fermentation based on LC-MS/MS. Microorganisms belonging to genera *Bacillus*, *Trichoderma* and *Aspergillus*, were capable of producing phytases and auxin derivatives. *B. subtilis* D strains cultivated in wheat bran, and *T. atroviride* (IOC 4503) cultivated in maize DDGS, produced the highest levels of indoles and phytases, respectively; therefore, they are promising for agricultural applications. Based on the present results, the production of indole derivatives by *Bacillus* and *Trichoderma* can be improved by substrates with low lignin content, such as soybean and wheat bran. Substrates at neutral pH and high hemicellulose concentrations are recommended for auxin production by *Bacillus*.

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