Original Paper

Production of α-amylase from Aspergillus flavus S2-OY using solid substrate fermentation of potato (Solanum tuberosum L.) peel

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Received: 17-05-2021 Accepted: 28-09-2021 Published: 30-10-2021

ABSTRACT

Amylases find commercial applications in diverse industries. This study investigated the production of α-amylase from a fungus, using potato peel as an alternative substrate. Six fungal isolates from the soil of cassava waste dumpsite were tentatively identified as two Aspergillus species and one species each of Rhizopus, Trichocladium, Neosartorya and Ascodermis. They were screened for their relative amylolytic activities using the starch agar hydrolysis test. Molecular characterization, using the ITS rRNA gene sequencing, was used to confirm the identity of the selected fungus as Aspergillus flavus S2-OY. Amylase production from the fungus via solid substrate fermentation of potato peel was carried out and the effect of production parameters investigated. Maximum α-amylase production was at an incubation period of 72 h, pH of 5.0 and temperature of 25 °C. Casein and maltose were the best nitrogen and carbon sources, respectively while the optima inoculum and moisture volumes for enzyme production were 1.5 mL and 5.0 mL, respectively. Aspergillus flavus S2-OY has great potential for α-amylase production using potato peel as an alternative and cheap substrate, under solid substrate fermentation condition. This has implications for the cost-effective production of an industrial enzyme and reduction of environmental pollution problems through effectual management of agro-industrial wastes.

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Keywords: Aspergillus flavus, α-amylase, optimization, solid substrate fermentation, potato peel, agricultural waste.

INTRODUCTION

Alpha-amylase (EC 3.2.1.1), also known as endo-1,4-α-d-glucanohydrolase, is an extracellular enzyme that randomly cleaves the 1,4-α-D-glycosidic bonds holding adjacent glucose molecules together, in a linear amylose chain in starch, thereby producing a range of products such as glucose, maltose and other oligosaccharides (Maity et al., 2015). Thus, the primary substrate of the enzyme is starch, which is one of the most abundantly distributed polysaccharides produced by plants, through the process of photosynthesis. Apart from α-amylase, other enzymes with the ability for starch degradation are β-amylase, glucoamylase, isoamylase and pullulanase (Hill et al., 2012).

Although, α-amylases are produced by plants, animals and microorganisms, the enzyme production from microorganisms are
preferred for commercial purpose due to benefits such as low cost, reliability, stability, less time and space needed for enzyme production (Simair et al., 2017). Therefore, the majority of industrial and biotechnological applications are derived from particular fungi and bacteria.

Alpha amylases are one of the most important enzymes in the field of biotechnology and enjoy approximately, 25% of the world enzyme market (Reddy et al., 2003) They find applications in various industries such as textile, confectionary, baking, brewing, sugar, alcohol, paper coating, starch, pharmaceutical and syrup industries (Oyeleke et al., 2013). Other uses include the use as digestive aid, detergents and for sewage treatment (Dhana et al., 2009).

Fruit and vegetable wastes resulting from agricultural and industrial processing, account for 30 to 50% of the input materials (Di Donato et al., 2011). The wastes are mostly generated from preliminary operations such as peelings and cuttings. Enormous amount of these wastes are disposed into the environment resulting into pollution hazards which are detrimental to its biological and physical components. Potato (Solanum tuberosum L.) is a major cash crop produced and sold all over the world. As a result of the processing of potato (Solanum tuberosum L.) into various products in industries, a lot of wastes are generated, chief amongst them is the potato peel, which is an epicarp covering of the potato root shielding its flesh (Shukla and Kar, 2006). These potato peels create severe disposal problems in industries as they are mostly discarded and allowed to rot causing environmental pollution. However, potato peels contain sufficient amount of nutrients for nutritional purposes and pharmacological use. Some these nutrients include dietary fiber, antioxidants, phenolic compounds, vitamins and minerals, and also cell wall could be used as substrates for fungal growth and α-amylase production (Shukla and Kar, 2006; Schieber et al., 2009). Increasing α-amylase production demands, high nutrient cost and environmental concerns have stimulated interest in the utilization of agrowastes as cost-effective substrates for production of the enzyme. Several agrowastes such as banana peels (Khan and Yadav, 2011), date wastes (Acourene and Ammouche, 2012), sugarcane baggase (Roses and Guerra, 2009) and rice husks (Oyeleke et al., 2013) were investigated as cost-effective substrates for α-amylase production.

Aspergillus species are useful microorganisms in food fermentations and biotechnological applications (Varga and Samson, 2008). Several strains of the species have been implicated in α-amylase production (Chimata et al., 2010; Kirankumar et al., 2011; Ajayi et al. 2014; Sethi et al., 2016). Optimization of fermentation parameters such as pH, temperature, incubation period, carbon and nitrogen sources, is one of the approaches employed for the achievement of high enzyme yield during the process (Sethi et al., 2017).

Alpha amylases are generally produced by using the submerged fermentation technique. However, lately, solid substrate fermentation method is gradually being utilized for this purpose (Xu et al., 2008; Zambare, 2010), due to advantages such as simple technique, superior and high volumetric productivity, low capital investment, low catabolite repression, marginal end-product inhibition, low energy requirement, simple fermentation equipment requirement, less water output and better product recovery (Ferreria et al., 2015).

The aim of this study was to optimize α-amylase production from Aspergillus flavus S2-OY under solid substrate fermentation condition, using potato peel as an alternative substrate. This was with a view for cost-effective production of this industrial enzyme and reduction of environmental pollution problems associated with the indiscriminate dumping of potato wastes.
MATERIALS AND METHODS

Fungal isolation and culture maintenance
Fungi were isolated from soil samples collected from the soil of cassava waste dumpsite. Distinct fungal growth on potato dextrose agar (PDA) plates were successively sub-cultured on fresh medium to obtain pure colonies. The pure fungal colonies were then maintained on potato dextrose agar (PDA) slants and stored at 4 °C.

Screening of fungal isolates for α-amylase production
The isolated fungi were screened for their relative α-amylase production capacity using the starch agar plate assay method of Ugo and Ijigbade (2013). The isolate with the highest zone of clearance was selected for further studies and maintained on potato dextrose agar slant at 4 °C.

Characterization and identification of fungal isolates
The isolated fungal isolates were characterized macroscopically and microscopically. The fungal morphology was studied macroscopically by observing their features on plate such as surface colour, margin, form, texture and diameter. Microscopic characterization was carried out using a compound binocular microscope to view the lactophenol cotton blue-stained slides mounted with a small portion of the mycelium. The characteristics observed were the spore type and shape, type of sporangia and type of hyphae (Gaddeyya et al., 2012). Molecular characterization was then carried out to confirm the identity of the selected fungus. This was based on the analysis of internal transcribed spacer (ITS) region of the ribosomal DNA gene (White et al. 1990). Fungal genomic DNA was extracted from 5-day old fungal culture with the aid of the ZR Fungal/Bacterial DNA MiniPrep kit (ZYMO RESEARCH, USA). Primers ITS 4 (F, 5’-TCCTCCGCTTATTGATATGS-3’) and ITS 5 (R, 5’-GGAAAGTAAAAGTCGTAACAGG-3’) were used to amplify the ribosomal gene. Amplification was carried out in a thermal cycler (Applied Biosystems) with reaction conditions: initial denaturation at 94 °C for 5 min, followed by 36 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, elongation at 72 °C for 45 s and followed by a final elongation at 72 °C for 7 min and hold temperature at 10 °C. The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer (Applied Biosystems).

Collection and preparation of agricultural wastes
Cassava (Manihot esculenta Crantz), potato (Solanum tuberosum L.) and yam (Dioscorea rotundata) peels were obtained from their respective processing sites in Ile-Ife, Nigeria. They were shredded and prepared by exhaustive washing with distilled water, oven-dried at a temperature of 60 °C for 1 week until they were well dried. Thereafter, the peels were milled and sieved into 0.5 mm particle sizes with milling machine. They were stored in clean, dried, airtight containers and kept in the refrigerator at 4 °C and were subsequently used as substrate in enzyme fermentation media (Ahmed et al., 2016).

Screening of agricultural wastes as substrate for α-amylase production
The powdered agricultural waste residues potato, yam and cassava peels were screened for their suitability as substrate for fungal growth and α-amylase production, under solid substrate fermentation condition. The crude enzyme was assayed for α-amylase activity. The agricultural waste which produced the highest activity was selected for use as substrate in the enzyme production process.

Proximate analysis of potato peel
Potato peel was selected due to its huge potential in supporting fungal growth and α-

Amylase production. The peel was chemically and nutritionally analyzed. The total carbohydrate, crude protein, lipid, crude fibre, moisture and ash contents were determined using the method described by AOAC (2005).

**Alpha amylase production**

Alpha amylase production using the solid-substrate fermentation technique was carried-out according to the modified method of Singh et al. (2009). The fermentation medium used for the production process contained 5 g of raw potato peel powder amended with 10 mL of minimal salt solution. The minimal salt solution contained the following; KH₂PO₄ 2.0 (g/l), MgSO₄.7H₂O 0.2 (g/l), NaCl 0.1 (g/l), CaCl₂ 0.1 (g/l) and MnSO₄ 0.5 (g/l) and peptone 0.2 (g/l) at pH 6.0. The culture medium was prepared in a series of 100 ml cotton-plugged Erlenmeyer flasks, homogenously mixed and sterilized at 121 °C for 15 min in an autoclave. Thereafter, the flasks were cooled at room temperature and each inoculated with 1.0 ml standardized fungal spore suspension (5.0 x 10⁵ spores/ml) of *Aspergillus* sp. under sterile condition, with the aid of sterile pipette. The flasks were then incubated at 30 °C for 5 days. At the end of fermentation, the fermented mass was mixed with 50 mL distilled water and agitated on a rotary shaker at 150 rpm for a period of 1 h. It was filtered using Whatman filter paper No. 1 and the filtrate was then centrifuged at 6000 rpm for 15 min. The cell-free supernatant was collected as crude enzyme for subsequent analysis.

**Alpha amylase assay**

α-Amylase activity was determined by estimating the amount of reducing sugars released in a reaction mixture containing 1.0 ml soluble starch in phosphate buffer, pH 6.0 and 1.0 ml of enzyme extract, according to the DNSA assay method as described by Miller (1959). Incubation was done at 50 °C in a water-bath for 10 min after which the reaction was terminated with 1.0 ml dinitrosalicylic acid (DNSA) reagent and the mixture boiled for 15 min. The test tubes were allowed to cool down and the absorbance read at 540 nm using a UV spectrophotometer. The concentration of the glucose released was read against the glucose standard. One unit (U) of enzyme activity was described as the amount of enzyme that released µmole of reducing sugar per minute per millilitre, under assay conditions.

**Optimization of α-amylase production**

**Effect of incubation period on α-amylase production**

This was carried out by using the method described by Puri et al. (2013). The effect of incubation period on α-amylase production and fungal growth, the fermentation medium was inoculated with selected fungus and incubated for 7 days. The flasks were incubated at 30 °C and was sampled every 24 h and assayed for enzyme production and the fungal growth estimated.

**Effect of pH on production of α-amylase**

The effect of pH on α-amylase production was determined by adjusting the pH of the fermentation medium to different levels 3.0 to 8.0. Thereafter, each flask containing the varied pH medium was inoculated with fungal spore suspension incubated for 72 h at 30 °C. After incubation, the enzyme activity was assayed.

**Effect of temperature on production of α-amylase**

The influence of temperature on α-amylase production was studied using a modified method of Puri et al. (2013). This was carried out by subjecting the fermentation medium to different temperature range 20 °C to 45 °C. The cell-free supernatant was obtained and enzyme production determined.

**Effect of carbon sources on production of α-amylase**

Different carbon sources - fructose, soluble starch, sucrose, glucose, lactose, maltose, and potato peel were investigated as
carbon sources for enzyme production. After incubation, the enzyme was extracted and the activity determined.

**Effect of nitrogen sources on α-amylase production**

The effect of different nitrogen sources, on α-amylase, were studied. These included casein hydrolysate, yeast extract, gelatin, tryptone, peptone, urea, ammonium nitrate and potassium nitrate. After incubation, the enzyme extract was assayed for its activity.

**Effect of inoculum sizes on α-amylase production**

The modified method of Puri et al. (2013) was used in studying the effect of inoculum sizes on α-amylase production. The fermentation medium was prepared and inoculated with different inoculum sizes 0.5 ml to 2.0 ml. After incubation, enzyme was extracted and assayed for its activity.

**Effect of moisture on α-amylase production**

The effect of moisture content on α-amylase production was studied. Different volumes of mineral salt solution used varied from 2.5 to 15.0 ml. The enzyme extract was assayed for α-amylase activity.

**Statistical analysis**

All data were subjected to statistical analysis for determination of mean and standard deviation using SPSS version 16. Experiments were carried out in triplicates.

**RESULTS**

**Isolation of fungi and screening for α-amylase production**

Six morphologically-distinct strains of fungi were isolated from soil samples collected from the cassava waste dumpsite at Tonkere, Ile-Ife. The isolates were successively subcultured on fresh PDA plates to obtain pure cultures. They were screened for α-amylase production using the starch agar hydrolysis assay and the strain Aspergillus sp. 1 with code (S2) exhibiting the largest zone of clearance was taken as that with the highest enzyme-production ability. It was therefore selected for further studies (Plate 1; Table 1).

**Characterization and identification of fungal isolates**

The fungal isolates were characterized based on their morphology on individual plates and under the microscope (i.e macroscopy and microscopy) (Table 2). The fungal isolates were identified based on the phenotypic characterization such as distinctive characters of the arrangement, shapes and sizes of macro- and microconidia, presence and absence of chlamydospores as well as cultural appearance and pigmention on potato dextrose agar medium. They were presumptively identified as two Aspergillus spp., and one species each of Rhizopus, Trichocladium, Neosartorya and Ascodermis. They were respectively maintained on PDA slants and stored at 4 °C. The selected amylolytic fungal isolate Aspergillus sp. 1 was molecularly characterized and identified based on ITS rRNA nucleotide sequencing and comparison to the sequences in NCBI database. The identity of the fungus was confirmed as Aspergillus flavus S2-OY with maximum identity of 97.47% to other Aspergillus flavus. The isolate was deposited in GenBank as the strain S2-OY (GenBank accession no. MZ267053.1).

**Screening of agricultural wastes for α-amylase production from A. flavus S2-OY**

Alpha amylase production was achieved with the use of the three agricultural wastes - yam, potato and cassava peels, as substrates, for the solid substrate fermentation. However, potato peel acted as the best substrate for α-amylase production with enzyme activity 30.13 ± 0.65 Units/ml. This was followed by the use of yam peel (20.07 ± 0.64 Units/ml) and cassava peel (14.60 ± 0.49 ml) as substrates (Figure 1).
Proximate analysis of potato peel

The result of the proximate analysis of potato peel is presented in Table 3. The potato peel contained 7.44 ± 0.14% moisture, 4.39 ± 0.17% ash, 6.16 ± 0.20% crude fibre, 9.37 ± 0.04% ether extract, 15.18 ± 0.13% crude protein, 57.56 ± 0.07% Nitrogen free extract and 63.91 ± 0.13% total carbohydrate.

Optimization of α-amylase production from fungus

Effect of incubation period on α-amylase production and fungal growth

The minimum enzyme activity of 41.45 ± 0.61 Units/ml was observed at 24 h. The production increased gradually until it reached a peak at 72 h (85.52 ± 0.50 Units/ml) of the fermentation period. This revealed that the optimum incubation period for α-amylase production from the selected fungus was 72 h of fermentation period whilst still in the exponential phase of fungal growth. Beyond this period, the enzyme production declined. However, the fungal growth increased exponentially with increase in incubation period until 120 h when it began to decline (Figure 2).

Effect of pH on α-amylase production

The α-amylase activity increased steadily from pH 3.0 (14.22 ± 0.57 Units/ml) until it reached a peak at pH 5.0 (40.07 ± 0.45 Units/ml). Thus, the optimum pH for the enzyme production was found to be 5.0. However, at pH values above this, there was a gradual reduction in α-amylase production (Figure 3).

Effect of temperature on α-amylase production

Alpha-amylase activity and production from A. flavus S2-OY increased from 20.49 ± 0.43 Units/mL at 20 °C incubation temperature to reach maximum at 25 °C (30.21 ± 0.50 Units/ml). α-Amylase production then decreased with increase in temperature down to a minimum value of 3.95 ± 0.37 Units/ml at 45 °C (Figure 4).

Effect of inoculum size on α-amylase production

Alpha-amylase production from A. flavus S2-OY, under solid substrate fermentation condition, was maximum when an inoculum size of 1.5 ml (79.03 ± 0.97 Units/ml) was used in the fermentation medium. α-Amylase production then decreased as inoculum size increased to 2.0 ml (60.04 ± 0.95 Units/ml (Figure 5).

Effect of moisture on α-amylase production

The effect of moisture on α-amylase production was also studied. Results revealed that the enzyme was most produced when the volume of 5.0 ml minimal salt solution (78.2 ± 0.68 Units/ml) was used for fermentation. Moisture volumes below and above this gave lesser enzyme production. Thus, the best moisture volume for α-amylase production from the fungus was 5.0 ml (Figure 6).

Effect of carbon sources on α-amylase production

Alpha-amylase production was maximum with the use of maltose as carbon source (66.25 ± 0.78 Units/ml). This was followed by the use of soluble starch (53.07 ± 0.73 Units/ml) and potato peel (48.14 ± 0.43 Units/ml) as carbon sources, while the lowest α-amylase production was achieved with the use of sucrose (26.98 ± 0.53 Units/ml) as carbon source for α-amylase production, under the solid substrate fermentation condition (Figure 7).

Effect of nitrogen sources on α-amylase production

Alpha-amylase production from A. flavus S2-OY was maximum (76.290.78 Units/ml) with the use of casein as nitrogen source. This was followed by the use of potassium nitrate (38.63 ± 0.50 Units/ml) as nitrogen source while the lowest activity was achieved with the use of gelatin (21.44 ± 0.68 Units/ml) as nitrogen source (Figure 8).
Plate 1 Zone of hydrolysis of leading Aspergillus sp. 1 on PDA plate at 30 °C, pH 5.6 after 5 days of incubation.

Table 1: Screening of fungal isolates for α-amylase-production by the starch hydrolysis plate assay.

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Fungal isolate</th>
<th>Halo zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Neosartorya sp.</td>
<td>2.39 ± 0.01</td>
</tr>
<tr>
<td>S2</td>
<td>Aspergillus sp.1</td>
<td>5.16 ± 0.04</td>
</tr>
<tr>
<td>S3</td>
<td>Trichocladium sp.</td>
<td>3.48 ± 0.03</td>
</tr>
<tr>
<td>S4</td>
<td>Rhizopus sp.</td>
<td>3.63 ± 0.08</td>
</tr>
<tr>
<td>S5</td>
<td>Aspergillus sp.2</td>
<td>3.52 ± 0.02</td>
</tr>
<tr>
<td>S6</td>
<td>Ascodermis sp.</td>
<td>2.76 ± 0.06</td>
</tr>
</tbody>
</table>
Table 2: Phenotypic characterization and identification of fungal isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Microscopic features</th>
<th>Presumptive Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Upright conidiosphore, terminating in a globose, radiating from the entire surface, I-celled.</td>
<td><em>Neosartorya</em> sp.</td>
</tr>
<tr>
<td>S2</td>
<td>Very long, thick, straight and upright conidiophores. Enlarged at the apex, radiating conidia from surface. Globose, ovoid, simple, bearing phialides at the head attached with conidia.</td>
<td><em>Aspergillus</em> sp. 1</td>
</tr>
<tr>
<td>S3</td>
<td>Conidiophores poorly developed, ovoid, septate.</td>
<td><em>Trichocladium</em> sp.</td>
</tr>
<tr>
<td>S4</td>
<td>Long conidiophores, bearing stolons, rhizoids, clustered apex with ripe conidia.</td>
<td><em>Rhizopus</em> sp.</td>
</tr>
<tr>
<td>S5</td>
<td>Long, thick, upright conidiosphore arising from the mycelium singly. Conidia head with phialides attached to it at its apex, I-celled, globose.</td>
<td><em>Aspergillus</em> sp. 1</td>
</tr>
<tr>
<td>S6</td>
<td>Ditch shape format, ovoid, globose, cluster conidia covered with a membrane.</td>
<td><em>Ascodermis</em> sp.</td>
</tr>
</tbody>
</table>

Figure 1: Screening of agricultural wastes as substrate for α-amylase production from *A. flavus* S2-OY.

Table 3: Proximate analysis of potato peel.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percentage Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>4.39 ± 0.17</td>
</tr>
<tr>
<td>Moisture</td>
<td>7.44 ± 0.14</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>6.16 ± 0.20</td>
</tr>
<tr>
<td>Ether extract</td>
<td>9.37 ± 0.04</td>
</tr>
<tr>
<td>Crude protein</td>
<td>15.18 ± 0.13</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>57.56 ± 0.07</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>63.91 ± 0.13</td>
</tr>
</tbody>
</table>
Figure 2: Effect of incubation period on fungal growth and α-amylase production.

Figure 3: Effect of pH on α-amylase production from A. flavus S2-OY.
Figure 4: Effect of temperature on $\alpha$-amylase production from *A. flavus* S2-OY.

Figure 5: Effect of inoculum size on $\alpha$-amylase production from *A. flavus* S2-OY.
Figure 6: Effect of moisture volume on α-amylase production from A. flavus S2-OY.

Figure 7: Effect of carbon sources on α-amylase production from A. flavus S2-OY.
DISCUSSION

In this study, six fungal strains were isolated from the soil of a cassava waste dumpsite at Tonkere village, Ile-Ife, Osun State, Nigeria. The six isolates were presumptively identified by phenotypic method as two *Aspergillus* species and one species each of *Rhizopus*, *Trichocladium*, *Neosartorya* and *Ascodermis*. Several workers had reported the occurrence of amylolytic fungi in the soil, especially of starch processing sites, describing the soil as a repository of amylase-producing microorganisms (Egbere et al., 2014; Ogbonna et al., 2014; Ferreira et al., 2015).

The fungal isolate with the most appreciable α-amylolytic activity was selected for further studies. It was presumptively identified as a strain of *Aspergillus* sp. 1. The identity of the selected fungus was confirmed by molecular characterization, using the ITS rRNA gene sequencing, as *Aspergillus flavus* S2-OY. Several strains of *Aspergillus* spp. have been implicated in α-amylase production (Ayanda et al., 2013; Ogbonna et al., 2014; Ali et al., 2017; Karim et al., 2018).

The most appreciable enzyme production was observed with the use of potato peel as substrate. There are several reports of the use of agricultural wastes as cheap substrates for microbial α-amylase production (Xu et al., 2008; Murthy et al. 2009; Jahir and Sachin, 2011). The selection of an agricultural waste as substrate for enzyme production depends upon several factors mainly related with cost and availability of the substrate. The

Figure 8: Effect of nitrogen sources on α-amylase production from *A. flavus* S2-OY.
solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as anchorage for the cells (Nimkar et al., 2010). Potato peel wastes contribute the major industrial food waste discarded in the environment, arising from potato processing and home wastes (Shukla and Kar, 2006; Schieber et al., 2009). Biotechnological applications of potato peel wastes are interesting not only from the point of view of low-cost substrate, but also in solving problems related to their disposal (Schieber et al., 2009).

The result of the proximate chemical analysis of the potato peel used in this study was similar to that of USDA (2008) that reported that in every 100 g of raw potato peel, the following nutrients can be found: 1.61 g ash, 0.1 g total fat, 2.57 g protein, 2.5 g total dietary fiber and 83.29 g water. However, a slight variation in the findings of the current research could be as a result of environmental conditions of the soil in which the potato tubers were grown.

Filamentous fungi are best adapted for solid substrate fermentation (SSF). The hyphal mode of fungal growth and their good tolerance to low water activity and high osmotic pressure conditions make fungi efficient and competitive in natural micro flora for bioconversion of solid substrates (Sethi et al., 2016). Traditionally, submerged fermentation has always been deployed for α-amylase production. However, solid substrate fermentation method is gradually being utilized for this purpose due to advantages such as simple technique, superior and high volumetric productivity, low capital investment, low catabolite repression, marginal end-product inhibition, low energy requirement, simple fermentation equipment requirement, less water output and better product recovery (Xu et al., 2008; Ferreria et al., 2015).

Alpha amylase production from the study fungus increased from 24 h and peaked at 72 h incubation period, after which there was a steady decline in enzyme production. A similar finding was reported by Nwagu and Okolo (2011), in which the test fungus also exhibited maximum extracellular amylase production after 72 h fermentation period. The decline in enzyme production was most likely due to reasons such as depletion of nutrients in the culture medium, production of other by-products of metabolism and enzyme denaturation (Sindhu et al., 2009; Haq et al., 2012). Fungal growth was observed to have continued even with the decrease in enzyme production within the period of incubation. This could be as a result of the availability of nutrients in the fermentation medium and the fungus still being in its exponential stage of growth.

Aspergillus flavus S2-OY exhibited maximum α-amylase production at pH 5.0. An optimum pH of 5.0 was similarly observed for α-amylase production from A. niger (Renato and Nelson (2009) and Penicillium purpurogenum (Silva et al., 2011). However, pH 5.5 was observed to be the optimum for α-amylase production from a strain of A. flavus (Ali et al., 2017). Hydrogen ion concentration (pH) plays important role in microbial growth by affecting enzyme secretion and product stability in the fermentation medium (Romero-Gomez et al., 2000).

Alpha amylase production was observed to be optimum at an incubation temperature of 25 °C while at temperatures above this, production declined and ceased at 45 °C. This result is similar to optimum temperature of 28 °C observed for α-amylase production from A. flavus (Ali et al., 2017). During enzyme production at differing temperatures, stabilization of metabolic network is supposedly by the folding of proteins of the metabolic network itself, most probably by acquiring chaperones (Borges and Ramos, 2005). Temperature above 45 °C results in moisture loss of the substrate, which affects metabolic activities of fungi, and results in reduced growth and α-amylase production (Sindhu et al., 2009).

The use of maltose as carbon source led to maximum level of enzyme production, followed by soluble starch and potato peel. Shalini and Majoj (2014) reported similar
finding for α-amylase production from Bacillus amyloliquifaciens. Carbon sources represent the energetic sources that are available for the growth of and metabolite production by microorganisms (Balkan et al., 2011).

Among the different nitrogen sources, casein gave maximum production of α-amylase from A. flavus S2-OY. The presence of organic nitrogen sources such as casein, urea and peptone has been reported to enhance αamylase enzyme production by microorganisms (Anto et al., 2006).

The best inoculum size for α-amylase production was 1.5 ml of fungal spore suspension. Balkan and Ertan (2010) reported that inoculum concentration 2.5 ml of Penicillium brevicompactum gave the maximum production of α-amylase. Higher inoculum concentration will increase the moisture content to a significant extent. The free excess liquid present in an unabsorbed form will therefore give rise to an additional diffusion barrier together with that imposed by the solid nature of the substrate and lead to a decrease in growth and enzyme production (Balkan et al., 2011). On the other hand, low inoculum size requires longer time for the cells to multiply to sufficient number to utilize the substrate and produce the desired product (Ramachandran et al., 2004). Further increase in inoculum sizes result in decreased enzyme synthesis, indicating that limitation of nutrients occurred due to the increased microbial activity.

Moisture is among the basic growth requirements of microorganisms. The most suitable moisture content needed for maximum α-amylase production in this study was 5.0 ml of minimal salt solution. The optimum moisture content for growth and substrate utilization depends upon the organism and water-holding capacity of substrate used for cultivation (Balkan et al., 2011).

Conclusion

The use of α-amylase in the food, baking, textile, detergent and pharmaceutical industries has been increasing with the growing demands of population. This study indicated the feasibility of the use of potato peel as an alternative substrate for α-amylase production from Aspergillus sp. via the solid substrate fermentation technique. Optimal conditions for maximum α-amylase production from the fungus, isolated from the soil of a cassava waste dumpsite, were incubation period of 72 h, initial medium pH of 5.0, temperature of 25 °C, casein and maltose as best nitrogen and carbon sources, respectively, as well as 1.5 ml inoculum size and 5.0 mL moisture content. This study has implications for the cost-effective production of a biotechnologically-important enzyme, as well as mitigation of environmental pollution problems through effectual management of agro-industrial wastes.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

Author OO conceived and monitored the stages of the research; Author VOA ran the laboratory procedures, did the literature search and statistical analysis; Author OO wrote the manuscript; Both authors read and approved the manuscript for publication.

ACKNOWLEDGEMENTS

Authors appreciate the staff of the Laboratory of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria, for provision of reagents and offer of required technical assistance for the study.

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