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Production and Characterization of Dextranase by *Penicillium brevicompactum* **Isolated from Garden Soil**

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Authors' contributions

Author SMW designed and supervised the study. Author OJI managed the analysis of the study, performed the statistical analysis and wrote the first draft. Author HAA edited and wrote the final manuscript. Authors SMW, HAA and OJI managed the literature searches. All the authors read and approved the final manuscript before submission.

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ABSTRACT

Aim: The aim of the study is to produce and characterize dextranase produced by fungi isolated from soil and honey.

Place and Duration of Study: The study was carried out at the Department of Microbiology, University of Ibadan, between April, 2017 and October, 2017.

Methodology: Garden soils were collected from different locations within the University of Ibadan, Nigeria, and filtered and unfiltered honey from a local honey farmer. Serially diluted soil and honey samples were pour-plated on PDA for isolation of fungi. Isolated fungi were screened, then on dextran containing medium to select dextranase producing strains.

Results: Forty two (42) fungi isolated from soil and honey was qualitatively and quantitatively screened for dextranolytic activity. Three moulds, *Penicillium brevicompactum* BG8*, Fusarium oxysporum* BG4 and *Alternaria alternata* DD5 were found to be the most potent dextranase producers with 18.01 DU/mL, 9.46 DU/ml and 8.26 DU/mL activities, respectively. Optimization of major parameters affecting enzyme production; including medium composition, pH, carbon

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(Temperature was not optimized at this stage) and nitrogen sources, substrate concentration revealed that maximum enzyme production was obtained when Pleszczynska medium was used for production. *Penicillium brevicompactum* BG8 and *Alternaria alternata* DD5 produced dextranase maximally at 1.0% dextran concentration with sodium nitrate and yeast extract as nitrogen sources at pH 5.5 at 30°C and 180 rpm for 5 days. *Fusarium oxysporum* BG4 produced dextranase maximally at 1.0% dextran concentration with sodium nitrate and yeast extract as nitrogen sources at pH 6.0 at 30°C and 180 rpm for 5 days. The production of dextranase by *Penicillium brevicompactum, Fusarium oxysporum* and *Alternaria alternata* increased to 65.41 DU/mL, 17.14 DU/mL and 20.10 DU/mL respectively when production was carried out using the best optimization conditions. *Penicillium brevicompactum* BG8 dextranase was purified to 14.1 fold homogeneity with an overall yield of 27% and an increase in specific activity from 2.67 – 37.68 U/g protein. *Penicillium brevicompactum* BG8 dextranase showed optimum activity at 50°C and pH 5.5, 2 mL substrate concentration and 1 ml enzyme concentration. Na⁺ ion activated dextranase while Cu²⁺ and Hg²⁺ ions completely inhibited the enzyme activity.

Conclusion: Soil and honey are potential sources of isolation of dextranase producing organisms, particularly in higher quantities which may probably provide a way out to cheap and commercially available enzyme.

Keywords: Dextran; dextranase; fungi; soil; honey.

1. INTRODUCTION

Dextran is a complex carbohydrate, that is, a chain of sugar molecules strung together. It is a long-chain, high-molecular-weight polymers that dissolve or disperse in water to give improved rheological properties. Dextran is a product of microbial action, majorly on sucrose. Dextran has a very wide variety of different molecular weight fractions. It varies from chains of very high molecular weight (> 2000 kDa) to chains of low molecular weight (3 kDa). Dextran is a biopolymer of great importance owing to its applications in the pharmaceutical, food, photo film manufacturing and fine chemical industries. There are several processes in the medicinal and clinical areas that involve the application of dextran. Dextran is used in the manufacture of blood plasma expanders, heparin substitutes for anticoagulant therapy, cosmetics, and other products [1-3]. Another use of dextran is the manufacture of Sephadex gels or beads, which are widely employed for industrial and laboratory protein separations [3]. The products of hyrolysis of dextran by dextranase are glucose, isomaltose and isomalto-oligosaccharide, thus the enzymes are commonly called glucanases (E.C. 3.2.1.11) [4].

Dextranases are of great significance due to its wide area of potential application. Dextranases find potential application in a number of industrial processes such as in the medical, food and pharmaceutical industries. Microbial dextranases have successfully replaced the chemical hydrolysis of dextran in dextran-hydrolysing processes. The spectrum of dextranase application has widened in many other fields, such as clinical, medical, and analytical chemistries, as well as their wide spread application in removal of dextran in sugar mills and in the reduction and prevention of dental caries [5]. In commercial sugar processing, the use of dextranase in the degradation of dextran has been shown to be the most suitable method of solving the problems of poor clarification and throughput in sugarcane juice, both of which are caused by the presence of dextran [6-8]. Due to the increasing demand for these enzymes in various industries, there is enormous interest in developing enzymes with better properties suitable for industrial applications and their cost effective production techniques [9]. They can be obtained from several sources, such as plants, animals and microorganisms. The dextranases of microorganisms have a broad spectrum of industrial applications, as microbes are easily manipulated to obtain enzymes of desired characteristics and more stable than when prepared with plant and animal dextranases [10]. However, research into successful application of a few number of commercially available microbial dextranases is continous since other methods of dextran hydrolysis in sugarcane processing are not technologically developed [11]; and for complete replacement of chemical hydrolysis of dextran in the clinical and medical industry. Dextranases have been derived from several fungi, yeasts and bacteria. However, enzymes from fungal sources have dominated applications in industrial sectors [12]. Species of *Penicillium, Chaetomium, Paecilomyces, Fusarium* and

Lipomyces are known to be good producers of dextranase. Moreover, *Chaetomium gracile* and *Penicillium funiculosum* have been widely used for commercial production of the enzyme [13]. The aim of this project is the production, characterization of dextranase, and investigation of some conditions which may boost its production by fungi isolated from soil and honey.

2. MATERIALS AND METHODS

2.1 Isolation and Screening of Microorganisms

Microorganisms capable of hydrolyzing dextran were isolated from various environments of garden soil and from filtered and unfiltered honey samples. Samples were serially diluted in sterile distilled water and 1 mL was taken from each of the 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶ dilutions and were plated on Potato Dextrose Agar for isolation of fungi using spread plate technique at 30 \pm 5°C for 3 - 7 days [14]. Distinct colonies were being harvested unto set plates containing screening medium A compounded with (w/v) 1.0% dextran, 0.2% KH_2PO_4 , 0.2% NaNO₃ and 0.2% yeast extract, 2% agar, pH 5.5 and incubated at 30 \pm 5°C. After 7 days, plates were flooded with Gram's Iodine solution to visualize hydrolytic zone around colonies capable of hydrolyzing dextran. Further screening was carried out in screening medium B compounded with (w/v) 1.0% dextran, 0.2% KH_2PO_4 , 0.2% NaNO₃ and 0.2% yeast extract, pH 5.5 and incubated at 30 \pm 5°C and 180 rpm for 5 days [15]. Highest enzyme producing fungi were identified on the basis of cultural and morphological analysis [16]. All media used were supplemented with streptomycin for control of bacterial contamination.

2.2 Biomass Production

The initial weight of Whatman No. 1 filter paper was determined before separation of the mycelium from the filtrate. After filtration, the filter paper containing the fungal mycelium was dried in a hot air oven at 70°C till a constant weight is achieved. The biomass produced by each fungal isolated was determined by subtracting the initial weight of the filter paper from the final weight of the filter paper and the mycelium. The dry weight of the biomass was determined as gram per 50 mL [17].

2.3 Culture Medium and Cultivation

The medium used for the enzyme production by all fungi consisted of (w/v) 1.0% dextran, 0.2% $KH₂PO₄$, 0.2% NaNO₃ and 0.2% yeast extract, pH 5.5 [5,17]. 50 mL aliquot was dispensed into 100 mL Erlenmeyer flasks, capped, sterilized and allowed to cool before use. Using a sterile cork borer, a mycelial plug (4 mm) was cut from a young cultured plate, dropped into the sterile liquid medium and shaken for proper dislodge of spores. Culture broth in 100 mL Erlenmeyer flasks was grown for 5 days at 30°C in a rotary shaking incubator (180 rpm). The content of each flask was filtered to separate the fungal mycelium. The filtrate was then clarified by cold centrifugation at 6000 rpm for 15 min [17]. The supernatant was decanted and used as crude enzyme. It was assayed for protein and enzyme activity.

2.4 Dextranase Assay

Dextranase activity was analysed with the 3,5 dinitrosalicylic acid (DNS) method adopting the procedure used by Kosaric et al. [18]. Cell free filtrate (1 mL) was incubated with 2.0 mL substrate (2.5% Dextran) solubilized in 0.1 M sodium acetate buffer (pH 5.4) at 40°C for 20 min. 3 mL of DNS reagent was added to stop the reaction and the mixture was heated in boiling water for 5 min to develop colour. The mixture was allowed to cool and the amount of reducing sugar liberated was estimated by measurement of the optical density at 540 nm. Enzyme activity was determined by measuring reducing sugar liberated during enzyme-substrate reaction [19].

One unit of dextranase (DU) was defined as the amount of enzyme required to catalyse the formation of 1 µmol of isomaltose per mL per minute at standard assay condition.

2.4.1 Protein determination

Quantitative estimation of proteins was determined by the method of Bradford [20] using bovine serum albumin as a standard. Cell free filtrate was incubated with 0.5 mL Coomassie Brilliant Blue dye and incubated in the dark at room temperature for 20 min. This is then read using a spectrophotometer at a wavelength of 595 nm and absorbance value recorded.

2.4.2 Selection of medium for dextranase production

Several reported media for dextranase production from the fungal isolates were used for higher enzyme production [5,12,18,21]. After inoculation, 50 mL of each medium was subjected to incubation on a rotary shaker for 5

days at 30°C and 180 rpm. After fermentation, broth was filtered and then centrifuged at 6000 rpm for 15 min at 4°C and enzyme activity was determined by standard assay procedure.

2.4.3 Optimization studies

The effect of carbon sources (glucose, sucrose, lactose, xylose, dextran); nitrogen sources (NaNO₃, yeast extract, peptone, urea, $KNO₃$, NaNO3+yeast extract); substrate (dextran) concentration (0.5%, 1%, 1.5%, 2%, 2.5%), initial medium pH (4.5, 5, 5.5, 6, 6.5) were investigated to optimize the culture conditions (Temperature was not optimized because previous work by the authors has revealed 30°C as optimum temperature) for maximum dextranase production by the selected fungal strains*.* After incubation period in each experiment, the culture filtrate was separated and assayed.

2.4.4 Partial enzyme purification

Crude dextranase from the culture supernatant was precipitated with solid ammonium sulfate. The precipitate was removed by centrifugation (6000 x g for 20 min.), dissolved in 0.1 M sodium acetate buffer, pH 5.4 and extensively dialysed against the same buffer for 24 hours at 4°C [22].

2.4.5 Enzyme characterization

Optimum temperature and pH were determined by changing individually the conditions of activity assays: Temperature $(20 - 80^{\circ}C)$, pH $(3 - 9)$, dextran concentration $(0.5$ mL $-$ 4 mL) and enzyme concentration (0.5 mL – 3 mL). Effects of different metal ions were also tested [12].

3. RESULTS AND DISCUSSION

A total of 42 fungi were isolated from garden soil and honey. Of the 42 isolated fungal strains, 30 were able to hydrolyse the dextran with varying degree of hydrolysis zones. Several reports have confirmed the isolation of dextranolytic microorganisms from deteriorated sugar cane samples, sugar cane baggases, molasses [14,23] and honey [24]. Seven (7) isolates which showed larger dextran hydrolytic zones on agar plates were screened in liquid medium. Three isolates with a considerable higher enzyme activities were selected, identified and used for further studies. *Penicillium brevicompactum* (18.01 DU/mL)*, Fusarium oxysporum* (9.46 DU/mL) and *Alternaria alternata* (8.26 DU/mL) are the most active dextranase producing fungi compared with other isolates (Table 1). For biomass production measured in dry cell weight in g/50 mL, *Penicillium brevicompactum* BG8 (0.92 g/50 mL) had the highest biomass and the least biomass was observed in isolate BG4 (0.13 g/50 mL) from garden soil.

3.1 Factors Affecting Dextranase Production

3.1.1 Media composition

Media reported by various researchers were used for optimal fungal growth and higher enzyme yield. Fungal growth was maximal when cultured in the Pleszczynska medium (Fig.1). The biomass weight of the fungal isolate was least in isolate BG4 and highest weight recorded in isolate BG8 in all the tested media. The average weight in the media ranged thus: Kosaric medium (0.11 g – 0.85 g); Pleszczynska medium (0.16 g - 0.92 g); Abdel-Aziz medium (0.12 – 0.83 g) and Sherief medium (0.09 – 0.52 g). Highest enzyme production was observed in the media reported by Pleszczynska et al. [5] for the three isolates and thereby was used for optimization and production of the enzyme. *Penicillium brevicompactum* BG8 (18.76 DU/ml); *Fusarium oxysporum* BG4 (9.74 DU/ml); *Alternaria alternata* DD5 (9.03 DU/ml). This is similar to the work done by Subasioglu and Cansunar [17] reporting production to be maximal in a medium with exact same components.

Table 1. Quantitative screening for dextranase production

Values were means of triplicate readings + Standard error (SE)

Fig. 1. Effect of medium composition on fungal growth and Dextranase production *BG4- Fusarium oxysporum; BG8- Penicillium brevicompactum; DD5-Alternaria alternate*

3.1.2 Effect of Carbon Source

A carbon source is essential for the growth and proliferation of a microorganism in a culture medium. All the carbohydrate sources used supported the growth of the isolates. *Penicillium brevicompactum* BG8 (0.63 g/50 mL - 0.91 g/50 mL) with highest biomass recorded in dextran; *Fusarium oxysporum* BG4 (0.09 g/50 mL - 0.17 g/50 mL) with highest biomass recorded in sucrose; *Alternaria alternata* DD5 (0.43 g/50 mL - 0.63 g/50 mL) with highest biomass recorded in lactose.

The highest dextranase activity was recorded when the medium was supplemented with dextran as the only carbon source. *Penicillium brevicompactum* BG8 (19.01 DU/mL); *Fusarium oxysporum* BG4 (9.44 DU/mL) and *Alternaria alternata* DD5 (9.88 DU/mL) (Fig. 2). This is similar to the work done by Sherief et al. [12] and Zohra et al. [14] reporting dextran as the only carbon source that supported dextranase production. This result clearly suggests that dextranase is an inducible enzyme and is also substrate specific. Some researchers have reported the extent of production of dextranase in the presence of dextrans of different molecular weights and also varying the production of dextranase in the presence of Sephadex (cross linked dextran) as substrates [18,23].

3.1.3 Effect of substrate concentration

The concentration of the substrate for induction of enzyme production was varied in the production medium (Fig. 3). Highest biomass production by *Penicillium brevicompactum* BG8 (0.83 g/50 mL – 0.95 g/50 mL) was recorded at a dextran concentration of 2.0% and 2.5%. *Fusarium oxysporum* BG4 (0.11 g/50 mL – 0.21 g/50 mL) had highest biomass at 1.0% dextran concentration while *Alternaria alternata* DD5 (0.41 g/50 mL $-$ 0.66 g/50 mL) had the highest biomass production at 1.5% dextran.

Penicillium brevicompactum BG8 (19.23 DU/mL)*, Fusarium oxysporum* BG4 (9.54 DU/mL) and *Alternaria alternata* DD5 (10.15 DU/mL), all had the highest enzyme production at a substrate concentration of 1.0%. This corroborates with studies carried out by Sherief et al. [12] and Zohra et al*.* [14] reporting that 1.0% dextran as substrate concentration had the highest dextranase production. It was found that another 0.5% increase in the dextran concentration resulted in gradual percentage decrease in enzyme production. Increase in substrate

concentration from 1% to 2% resulted in approximately 7.5% decrease in enzyme approximately 7.5% decrease production. This could be as a result of limitation of fungal growth due to the increased viscosity of the culture medium which is an implication of

increased concentration of dextran in the culture medium. Mahmoud et al. [25] reported 3.5% as the substrate concentration supporting maximum enzyme production.

Fig. 2. Effect of carbon source on fungal growth and Dextranase production *BG4- Fusarium oxysporum; BG8- Penicillium brevicompactum; DD5-Alternaria alternata*

Fig. 3. Effect of substrate concentration on fungal growth Dextranase production *BG4- Fusarium oxysporum; BG8- Penicillium brevicompactum; DD5-Alternaria alternate*

Fig. 4. Effect of nitrogen source on fungal growth and Dextranase production *BG4- Fusarium oxysporum; BG8- Penicillium brevicompactum; DD5-Alternaria alternate*

3.1.4 Effect of nitrogen source

The nitrogen source present in a culture medium can greatly increase or reduce enzyme production and microbial growth in such medium. *Penicillium brevicompactum* BG8 (0.92 g/50 mL), *Fusarium oxysporum* BG4 (0.20 g/50 mL) and *Alternaria alternata* DD5 (0.58 g/50 mL) recorded the highest biomass in the control medium (Fig. 4). The control medium is a medium containing a combination of yeast extract and sodium nitrate as nitrogen source.

Maximum enzyme production was achieved in the control medium for *Penicillium brevicompactum* BG8 (19.10 DU/mL)*, Fusarium oxysporum* BG4 (9.56 DU/mL) and *Alternaria alternata* DD5 (10.72 DU/mL). This is similar to the work done by Fukumoto et al. [26] and Abdel-Naby et al. [27]. This result suggests that a single nitrogen source is insufficient for organismal boom and thereby higher enzyme yield. This result also confirms the composition of the production medium as the best as the two nitrogen sources were both present in it. Sherief et al. [12] reported ammonium dihydrogen phosphate as the best nitrogen source for dextranase production. Zohra et al. [14] also reported a combination of nitrogen sources

(yeast extract and peptone) for the production of the enzyme.

3.1.5 Optimization of medium pH

The growth of *Penicillium brevicompactum* BG8 within the pH ranges of $4.5 - 6.5$ varied from 0.65 g/50 mL – 0.92 g/50 mL with the highest biomass production (0.92 g/50 mL) at pH 5.5. *Fusarium oxysporum* BG4 had a growth rate ranging from 0.10 g/50 mL – 0.23 g/50 mL with the highest biomass (0.23 g/50 mL) at pH 6.0. *Alternaria alternata* DD5 growth ranged from 0.42 g/50 mL $-$ 0.60 g/50 mL and the highest biomass production recorded when pH of culture medium was adjusted to 5.5 (Fig. 5).

Dextranase production for *Penicillium brevicompactum* BG8 was maximal (19.12 DU/mL) at pH 5.5. *Fusarium oxysporum* BG4 maximum dextranase production (10.25 DU/mL) was at pH 5.5, while *Alternaria alternata* DD5 had the highest activity (8.94 DU/mL) at pH 6. This is in agreement with the research carried out by Sherief et al. [12] reporting pH 5.5 as the pH for maximal enzyme production by *Aspergillus subolivaceous* and *Pythium* sp. and pH 6.0 as the optimum pH for production by *Aspergillus terreus.* Das and Dutta [28] and

Shimizu and others [29] also reported pH for the production of dextranase from fungi to be optimal at $5.5 - 6.0$. Zohra et al. $[14]$ reported the optimum pH for the production of dextranase from a bacterial species also to be 6.0. On the contrary, some researchers have reported production of alkalophilic dextranase from *Catenovulum* sp. DP03, *Streptomyces* sp. NK458 and *Bacillus subtilis* NRC-B233b with their maximum activities occurred at pH 8.0, 9.0 and 9.2, respectively [8,24,30].

Table 2 shows dextranase production by fungi under optimized conditions. *Penicillium brevicompactum* BG8 had the highest growth with 1.52 g/50 mL biomass dry cell weight and enzyme activity of 65.41 DU/mL which is much higher than dextranase production by other fungi. This result could instigate further research into dextranase produced by the fungus and the feasibility of the commercial usage of the dextranase produced by this fungus.

3.2 Partial Enzyme Purification

The crude enzyme was partially purified using the ammonium sulphate salt. Enzyme was precipitated out to a level of 100% and suspended in 0.1M sodium acetate buffer pH 5.4. Dextranase enzyme from *Penicillium*

Fig. 5. Effect of medium pH on fungal growth and Dextranase production *BG4- Fusarium oxysporum; BG8- Penicillium brevicompactum; DD5-Alternaria alternate*

brevicompactum BG8 was purified to 14.1 fold to homogeneity with an overall yield of 27% and an increase in specific activity from 2.67 U/g to 37.68 U/g protein (Table 3).

3.3 Enzyme Characterization

3.3.1 Effect of enzyme and substrate concentration on Dextranase activity

Dextranase activity was optimum when 1.0 mL of the enzyme was used followed by a sharp reduction in dextranase activity with a gradual reduction from 2.0 mL of the enzyme concentration (Fig. 6). Activity of the dextranase produced by *Penicillium brevicompactum* BG8 increases with increase in substrate concentration till 2 mL, followed by a decrease up till 3 mL with a slight increase after (Fig. 7). Highest enzyme (80 DU/ml) activity was recorded at a substrate concentration of 2 mL.

3.3.2 Effect of temperature and pH on dextranase activity

Dextranase was optimally active at 50°C and pH 5.5 (Figs. 8 and 9). This is contrary to the work done by Raíces et al. [31] who recorded optimal activity of *Penicillium minioluteum* at pH 4.5 – 5.0 and a temperature of 35°C. Wanda and Curtiss [32] reported the recombinant enzyme of *Streptococcus sobrinus* presented a maximal activity at pH 5.3 and 39°C. Szczodrak et al. [33] reported dextranase produced by a strain of the fungus *Penicillium notatum* to be relatively stable in the raw stage and reached a maximal activity at pH 5.0 and 50°C. *Thermoanaerobacter wiegelii* dextranase reached a maximal activity at pH 5.5 and 70°C [34]. Mahmoud et al. [25] also recorded maximum activity of *Penicillium aculeatum* at pH 4.5 and 45°C.

Fig. 6. Effect of enzyme concentration on *Penicillium brevicompactum* **BG8 dextranase**

Fig. 7. Effect of substrate concentration on *Penicillium brevicompactum* **BG8 dextranase**

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Fig. 8. Effect of temperature on *Penicillium brevicompactum* **BG8 dextranase**

Fig. 9. Effect of pH on *Penicillium brevicompactum* **BG8 dextranase**

Fig. 10. Effect of metal ions on *Penicillium brevicompactum* **BG8 dextranase**

3.3.3 Effect of metal ions on dextranase activity

Penicillium brevicompactum BG8 dextranase activity was enhanced by $Na⁺$ and $K⁺$. The dextranase was partially inhibited by Mg^{2+} , Fe²⁺ and Zn^{2+} and was completely inactivated by Cu²⁺ and Hg²⁺(Fig. 10). The inhibitory effect of Fe²⁺ on dextranase activity observed is similar to the work done by Mahmoud et al. [25] where $Fe²⁺$ and $Fe³⁺$ were found to be strong inhibitors of dextranase activity. The inhibitory effect of $Mg²⁺$ on dextranase activity observed is contrary to the work done by Sherief et al. [10] who reported Aspergillus subolivaceous dextranase to be activated by Mg^{2+} and Mahmoud et al. [25] who reported Mg^{2+} has no effect on dextranase activity. Complete inactivation of the enzyme by Hg^{2+} is similar to the report of Das and Dutta [28] who reported that dextranase from *Penicillium lilacium* was inactivated by Hg^{2+} .

4. CONCLUSION

This study has confirmed that the *Penicillium* spp. are capable of producing dextranase and in very high quantities. *Penicillium brevicompactum* BG8 has the ability to produce dextranase under agitation (180 rpm) conditions at pH 5.5, temperature 30°C and 5 days incubation time, 1.0% dextran as carbon source, sodium nitrate and yeast extract as nitrogen source. Dextranase of *Penicillium brevicompactum* could therefore be suitable as an enzyme in technological process.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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