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Screening of Fungi Species Isolated from Fermented Milk for Extracellular Beta-galactosidase Production Potentials

[1,3]Idris, A. D.[*], [1]Abalaka, M. E., [1]Oyewole O. A., and [2]Evans, E. C.

[1]Department of Microbiology, Federal University of Technology Minna, Niger State Nigeria. [2]Department of Biochemistry, Federal University of Technology Minna, Niger State Nigeria. [3]Department of Biological Sciences, The Federal Polytechnic Bida, Niger State Nigeria.

aidabban@gmail.com

Abstract

An attempt was made to isolate and screen fungal species from fermented cow, sheep and goat milk for their potential to produce extracellular beta-galactosidase. Samples of fresh cow, sheep and goat milk were collected and allowed to ferment spontaneously. Thereafter, fungal species were isolated and characterized from the fermented milk samples using standard mycological techniques. The fungal isolates were further screened for qualitative and quantitative beta-galactosidase production using Yeast Extract Peptone (YEP) broth method. Selected fungal strain with the best beta-galactosidase production potential was identified using molecular technique. A total of 14 fungi strains were isolated from the fermented milk samples including *Aspergillus niger* (21.4%), *Aspergillus flavus* (21.4%), *Geotrichum candidum* (14.3%), *Penicillium* sp. (14.3%), *Candida* sp. (14.3%), *Aspergillus fumigatus* (7.1%) and *Aspergillus terreus* (7.1%). Qualitative screening of the fungal isolates for beta-galactosidase production showed *Aspergillus niger* FGMTF1, *Aspergillus niger* FSMTF6 and *Geotrichum candidum* FSMTF2 were positive for the enzyme production. Further quantitative screening of the fungal isolates revealed *Aspergillus niger* FGMTF01 to be the highest beta-galactosidase producer with enzyme activity of 19.60U/ml followed by *Geotrichum candidum*

FSMTF2 and *Aspergillus niger* FSMTF6 with enzyme activities of 18.55U/ml and 18.22U/ml respectively. Molecular identification confirmed the best enzyme producing fungal isolate to be *Aspergillus niger*. These organisms can be potential candidates for large scale production of beta-galactosidases. All the fungal isolated from this investigation can also be screened for production of other industrially important metabolites.

Keywords: Beta-Galactosidase, Fungi, Isolation, Screening, Fermented Milk

INTRODUCTION

Beta-D-galactosidase (EC 3.2.1.23, Beta-galactosidases, galactohydrolase, lactase, beta-gal or β-gal) is a glycoside hydrolase enzyme that catalyzes two different reactions (Panesar *et al*., 2016; de Campos *et al*., 2023). One is the hydrolysis of β-galactosides into monosaccharides glucose and galactose through the breaking of a glycosidic bond (Volford *et al*., 2021; Li *et al*., 2023). The second is a process called transgalactosylation to make allolactose, which creates a positive feedback loop for the production of β-galactosidase (de Albuquerque *et al*., 2021; Lutz-Wahl *et al*., 2024). Beta-galactosidase has also been reported to cleave fucosides and arabinosides but with much lower efficiency (Dahal *et al*., 2020). It is an essential enzyme in the human body for the digestion of bovine milk which contains an average of 4.8% lactose (Zaman *et al*., 2023).

Beta-galactosidase is important for organisms as it is a key factor in the production of energy and a source of carbons through the breakdown of lactose to galactose and glucose (Martarello *et al*., 2019). It is one of the most important enzymes used in the food industry, which offers nutritional, technological and environmental applications (Panesar *et al*., 2016). The enzyme also has many industrial, biotechnological and medicinal applications like cleavage of blood group A and B glycotopes, biosensors for lactose determination and enzymatic hydrolysis of lactose (Singh and Sambyal, 2023). Hydrolytic activity of t his enzyme is important in the food industry as it is used in the reduction of lactose content in milk, hence increasing the solubility and sweetness in the dairy products (Kocabaş *et al*., 2022). The enzyme is used in dairy products such as yoghurt, sour cream, and some cheeses

which are treated with the enzyme to break down any lactose before human consumption (Shafi and Husain, 2022).

Beta-galactosidase may be found in nature, particularly plants such as almonds, peaches, apricots, apples, tomatoes, certain species of wild roses; animal organs such as the intestine, brain, placenta, testis and skin tissues; and microorganisms (Panesar *et al*., 2016; Kaur *et al*., 2023). Beta-galactosidase can be synthesized from many sources including animals, plants, bacteria, filamentous fungi, and yeast (Volford *et al*., 2021). Microorganisms especially fungi are producers of this important enzyme using lactose as the sole source of carbon and energy (Ahmed *et al*., 2016).

A widely diverse population of different groups of microorganisms has been reported to produce this important enzyme. They include yeasts, e.g. *Kluyveromyces lactis*, *K. fragilis K*. *marxianus*, *Candida kefyr* and *Candida pseudotropicalis*; bacteria, such as *Escherichia coli*, *Lactobacillus bulgaricus*, *Streptococcus lactis, Pyrococcus woesei*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Enterobacter cloacae*, *Geobacillus stearothermophilus*, *Thermus sp.* and *Bacillus* sp; and moulds, such as *Aspergillus foetidus*, *A. niger*, *A. oryzae*, *A. Phoenecia*, *Alternaria alternata*, *Trichoderma reesei, T. viride, T. harzianum*, *Penicillium simplicissimum,* and *Penicillium expansum* (Asraf and Gunasekaran, 2010; Kaur *et al*., 2023).

A major challenge faced by industrial microbiologists is the choice and use of highly selective screening techniques for detection and selection of fungi of interest from the otherwise high microbial population with great potential for beta-galactosidase production (Casida, 2018). The production of microbial enzymes including beta-galactosidase is highly influenced primarily by the type of microbial strain used, physical parameters of fermentation and the composition of the growth medium (Ahmed *et al*., 2016). In view of the above, this study was carried out to isolate fungal strains from fermented milk samples and screen same for extracellular beta-galactosidase production potentials.

MATERIALS AND METHODS

Study Area

This study was conducted in the Department of Microbiology, Federal University of Technology Minna, Niger State Nigeria.

Collection of Sample

Samples of cow, sheep and goat milk were collected from production points within Minna, Niger State Nigeria. The samples were collected in clean sample containers and transported immediately to the Laboratory. The samples were kept at room temperature to allow spontaneous fermentation.

Isolation and Characterization of Fungi

Preparation of media

Potato Dextrose Agar (PDA) was prepared according to manufacturer's instruction, supplemented with lactose (40g/l) as substrate and chloramphenicol to inhibit bacterial growth. The media was sterilized at 121ºC for 15minutes in an autoclave, poured aseptically into petri-plates and Bijou bottles and incubated at room temperature for 24hours to ensure sterility.

Cultivation of fungi

Using direct plating technique, 0.5mL of fermented milk samples were aseptically inoculated on the prepared PDA and spread evenly by gently rotating the plate. All inoculated plates were incubated at (30ºC) for 5-7days in an incubator. Organisms obtained after the incubation period were sub-cultured to obtain pure fungal isolates. Stock cultures of the isolates were obtained by sub-culturing the pure isolates on agar slants and stored for future use (Walsh *et al*., 2018).

Characterization and identification of fungal isolates

The fungal isolates were characterized and identified based on macroscopic and microscopic analysis using taxonomic guidelines and standard procedures as described by Walsh *et al*. (2018). Macroscopic characteristics of 5-7day old fungal isolates was examined such as colonial growth, colonial color, presence or absence of aerial mycelium, nature of mycelia, texture, growth pattern, presence of wrinkles and furrows, pigment production. Microscopic examination was carried out using Lactophenol cotton blue (LPCB) staining and culture slide technique as described by Barnett and Hunter (1999) and Walsh *et al*. (2018).

Determination of percentage frequency of occurrence of fungal isolates

The percentage frequency of occurrence for each fungal species isolated from the fermented milk samples was determined as described by Sampo *et al.* (1997) and

Makut and Owolewa (2011). This was computed using the formula: A/B x 100; where A = Number of a particular fungal strain; and B = Total number fungi isolated from the sample.

Screening of Fungal Isolates for Potential to Produce Beta-galactosidase

Qualitative Screening

Production of beta-galactosidase was determined in all the fungal isolates by assessing their ability to utilize lactose using the Yeast Extract Peptone Lactose Broth (YEPLB) method as described by Owuama (2015) and Dahal *et al*. (2020). YEPLB (g/L): (Yeast extract-10, Peptone- 20, Lactose- 20, Chloramphenicol- 0.1, Phenol red-0.02) was prepared in Erlenmeyer flasks, boiled and about 12ml were transferred into test tubes. The test tubes containing the media were sterilized in an autoclave at 121°C for 15min. The media were allowed to cool and the test fungal isolates were inoculated into the test tubes containing the media. Thereafter, the inoculated tubes were incubated at 37°C for 7 days. The tubes were observed daily for change in colour from red to yellow which indicates production of the enzyme. Positive isolates were selected for further analysis.

Quantitative Screening

Preparation of Inoculum

The selected fungal isolates was sub-cultured on SDA slants and incubated at room temperature for 5days. Using a sterile syringe, 5.0mL of 2.5% sterile Tween 80 was aseptically dispensed into the SDA slants containing the fungal isolates. The spores were dislodged by shaking and 1mL of the spore suspension was diluted in 99ml of sterile distilled water to give an inoculum size of 1.0 x 10^6spores per mL. This was determined by counting in Neubauer chamber (Adhikari and Shrestha, 1989). This was used as inoculum for the fermentation experiment (Antoine *et al*., 2015).

Production of beta-galactosidase by selected fungal isolates

Lactose broth (containing g/L: Lactose - 20.0, $NaNO_3$ - 2.0, K_2HPO_4 - 1.0, KCl - 0.5, $MgSO_4$. $7H_2O$ - 0.5, $FeSO_4.5H_2O$ - 0.01) pH 6.8 was prepared in 500 mL Erlenmeyer flasks according to manufacturers' instruction and sterilized at 121ºC for 15minutes in an autoclave. Using a sterile syringe, 2.5mL of the inoculum was aseptically

inoculated into 500mL Erlenmeyer flask containing 250mL of the sterile lactose broth. The broth was incubated at room temperature for 7days (Akinola *et al*., 2012). After incubation period, the broth was filtered through a double layered muslin cloth and the filtrate was centrifuged at 10000 rpm for 10minutes at 4^{o}C. The supernatant was separated from the pellet using a clean Pasteur pipette into clean test tubes. The solution was tested for beta-galactosidase activity (Murugan, 2013).

Determination of enzyme activity

Using a micropipette, about 750µl of 2.0mM ONPG (Sigma-Aldrich) substrate solution (pH 6.8) was added to a series of test tubes and 250µl of crude enzyme were subsequently added in the test tube. Thereafter, 2ml of phosphate buffer (pH 6.8, 0.1 M) was added to the test tube and then incubated at 37ºC for 70 minutes. The reaction was stopped by addition of 250µl of 0.5M Na_2CO_3. Reaction progress was determined by estimating the O-nitrophenol (ONP) liberated at a wavelength of 420 nm against the blank (750 µl ONPG, 2250 µl phosphate buffer of pH 6.8) using a spectrophotometer. The activity of the enzyme was afterwards calculated using the formula (Dahal *et al*., 2020).

Beta-galactosidase activity (U/ml) $= \dfrac{A \times T_{rv}}{E_v \times T}$

Where; A = Absorbance at 420nm; Trv = Total reaction volume; E_v = Enzyme volume; T = Incubation time. One unit of beta-galactosidase activity is defined as the amount of the enzyme required to liberate 1µmole of O-nitrophenol (ONP) per minute under assay conditions (Antoine *et al*., 2015; Panesar *et al*., 2016). The fungal strains with the highest beta-galactosidase activities were selected for further studies.

Molecular Identification of Selected Fungal Isolates

At the molecular level, the selected fungal isolate was identified *via* DNA extraction, polymerase reaction, gel electrophoresis, integrity testing, purification of the amplified product and sequence analysis as described by Wawrik *et al.* (2005) and Frank *et al.* (2008). The nucleotide sequence of the fungal isolate was inserted in the NCBI Basic Local Alignment Search Tool (BLAST) for similar nucleotide sequence and genetic relatedness with other fungal isolates.

Data Analysis

The data obtained were expressed as Mean ± SD of triplicate determination (n=3).

RESULTS AND DISCUSSION

Cultural and Microscopic Characteristics of Fungi Isolated from Fermented Milk

A total 14 fungal species which consists of 10 moulds and 4 yeasts were isolated from the fermented milk samples. Filamentous fungi encountered in this study include *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium* spp., and *Aspergillus fumigatus*. Meanwhile the yeasts isolated from the samples were *Candida* spp., and *Geotricum candidum*. Tables 1a and 1b summarize the cultural and microscopic characteristics of the fungal isolates.

The fourteen fungal species isolated from the fermented milk samples may have originated from the atmosphere, the handlers, the udder or skin of the animals. The presence of these organisms is an indicator that these fungi are able to survive the various intrinsic factors of the milk samples including lysozyme and other lytic enzymes present in the milk samples; the acidic (low) pH of the fermented samples due to the accumulation and subsequent high concentration of lactic acid present in the sample; as well as the sole presence of lactose as the only metabolizable sugar present in the sample. It is very well known that very few microorganisms have the ability to metabolize lactose sugar. In view of these, the fungal species encountered in these samples may have potential applications such as beta-galactosidase and other dairy related enzyme production, lactic acid production as well as other industrial and biotechnological applications.

This result is in agreement with the findings of Omola *et al*. (2019), Yusuf *et al*. (2020), Nassarawa and Sulaiman, (2021) and Ogwaro *et al*. (2023) in their separate studies reported the presence of similar fungal species in various fresh and fermented milk samples in Kano and Kebbi States Nigeria, as well as Western Kenya. Meanwhile, this finding disagrees with the results of Qvirist *et al*. (2016) (Yaghnob Valley, Tajikistan), Fagbemigun *et al*. (2021) (Bauchi, Jigawa, Kano and Katsina States in Northern Nigeria), Pei *et al*. (2021) (China) and Ding *et al.* (2022) (Qinghai, Tibetan plateau) who reported totally different species of fungi in fresh and fermented milk samples including *Kluyveromyces marxianus*, *Kazachstania unispora*,

Cladosporium sp., *Ramularia* sp., *Actinomucor* sp. *Saccharomyces cerevisiae*, *Pichia kudriavzevii* and *Debaryomyces hansenii*. The disagreement could be attributed to difference in environmental conditions, season or time of investigation, nature of the samples or methodology used.

Frequency of Occurrence of Fungi Isolated from Fermented Milk

The percentage occurrence of the fungal groups isolated from the samples is shown in Figure 1. The highest occurring fungi encountered in the samples were *Aspergillus niger* and *Aspergillus flavus* both having percentage occurrence of 21.40% each, followed by *Penicillium* sp., *Candida* sp., and *Geotricum candidum* each with a percentage occurrence of 14.30%. The least encountered fungi were *Aspergillus terreus* and *Aspergillus fumigatus* with percentage occurrence of 7.10% each.

The high frequency of occurrence encountered for species of *Aspergillus* in the fermented milk samples could be attributed to the abundance and hardiness of their spore in the atmosphere. The spores of these organisms remain viable in the atmosphere and can easily settle on skin of man and animals as well as contaminate food materials. The abundance of spores of *Aspergillus* species in the atmosphere makes them easily obtainable with several positive consequences especially in natural ecosystems and many industrial applications.

Table 1a: Characteristics of Fungi Isolated from Fermented Milk

S/No	Code	Cultural Characteristics	Microscopic Characteristics	Inference
1	FGMTF01	Black surface with white border, deep thallus. Reverse creamy white.	Septate hyphae, long and smooth conidiophores. Biseriate phialides radiates around entire vesicles. Conidia is rough and dark	*Aspergillus niger*
2	FGMTF03	Green surface with white borders, speck of yellow can be observed. Cotton to velvety texture. Reverse is milky	Septate hyphae, long conidiophores with rough spiny apex walls. Phialides are uniseriate and biseriate over vesicles. Loosely radiated phialides with columns of conidia. Smooth to slightly rough conidia walls	*Aspergillus flavus*

3	FGMTF04	Surface is green in colour with white borders. Slight specks of yellow present. Cottony to velvety texture, reverse is milky	Septate hyphae, long conidiophores with rough spiny apex walls. Phialides are uniseriate over vesicles. Loosely radiated phialides with columns of conidia.	*Aspergillus flavus*
4	FGMTF05	Deep black surface, white border, deep thallus, reverse is milky black	Septate hyphae, long and smooth conidiophores. Biseriate phialides radiates around entire vesicles. Conidia is rough and dark	*Aspergillus niger*
5	FGMTF06	Surface is tan brown, velvety to powdery texture. Reverse is tan.	Septate hyphae, smooth and short conidiophores. Phialides are biseriate with equal length of phialides and metulae. Round and smooth conidia	*Aspergillus terreus*
6	FGMTF08	Surface is light green to grey in colour with white borders. Slight specks of yellow present. Cottony to velvety texture, reverse is milky	Septate hyphae, long conidiophores with rough spiny apex walls. Phialides are uniseriate over vesicles. Loosely radiated phialides with columns of conidia.	*Aspergillus flavus*
7	FGMTF09	Surface is white at first, then develops to dark green with specks of white cotton-like surface and white thick border. Fast growing. Reverse is milky brown	Septate hyphae with with branched and unbranched conidiophores that have secondary branched metuale. Metulae are arranged in whorls. Phialides are flask-shaped and bears unbranched chains of smooth and round conidia. The entire structure has a brush appearance	*Penicillium* spp

Table 1b: Characteristics of Fungi Isolated from Fermented Milk

S/No	Code	Cultural Characteristics	Microscopic Characteristics	Inference
8	FSMTF01	White moist colonies, hypahe are peripheral with ground glass	Gram stain reveals coarse false hypahe with rectangular arthroconidia segments that	*Geotricum candidum*

		appearance with short white aerial mycelium	varies in length with slight roundness at ends	
9	FSMTF02	White moist colonies, hypahe are peripheral with ground glass appearance with short white aerial mycelium	Gram stain reveals coarse false hyphae with rectangular arthroconidia segments that varies in length with slight roundness at ends	*Geotricum candidum*
10	FSMTF04	Slow growing with initial white surface which develops to a deep green colour with a thin white border. Reverse is brown.	Septate hyphae with branched and unbranched conidiophores that have secondary branched metuale. Metulae are arranged in whorls. Phialides are flask-shaped and bears unbranched chains of smooth and round conidia. The entire structure has a brush appearance	*Penicillium* spp
11	FSMTF06	Deep black surface, white border, deep thallus, reverse is milky black	Septate hyphae, long and smooth conidiophores. Biseriate phialides radiates around entire vesicles. Conidia is rough and dark	*Aspergillus niger*
12	FCMTF01	Creamy white, rough odd shaped colonies	Gram stained cells are oval and cigar in shape. Presence of budding	*Candida* spp
13	FCMTF02	White to creamy, smooth, pasty colonies, odd shaped rough colonies	Gram stained cells are oval and cigar in shape. Presence of budding	*Candida* spp
14	FCMSV02	Surface is velvety with various shades of green and white border. Reverse is milky	Septate hyphae with short but smooth conidiophores. Compact uniseriate phialides. Round smooth and slightly rough condia. Phialides are columnar	*Aspergillus fumigatus*

Keys: FGMTF – Fermented Goat Milk Tudun Fulani; FSMTF – Fermented Sheep Milk Tudun Fulani; FCMTF - Fermented Cow Milk Tudun Fulani; FCMSV – Fermented Sheep Milk Shanu Village

The findings of this study is in agreement with the research of Omola *et al*. (2019) and Yusuf *et al*. (2020) who in their separate investigations in Kano and Kebbi States respectively in Nigeria reported species of *Aspergillus* as the most abundant fungal group present in fermented milk samples. However, the investigations of Nassarawa and Sulaiman (2021) in Kano State Nigeria and Pei *et al*. (2021) in China disagrees with the findings of this study as they reported in their separate studies *Mucor* spp. and *Cladosporium* spp. as the highest occurring group of fungi found in fermented milk samples with percentages occurrences of 50% and 30% respectively. This disagreement could be attributed to the difference in some factors such as time or season of the research, nature of the sample, environment or methodology used.

Beta-galactosidase Production Potentials of Fungal Isolates

Colour change observed in the YEPLB tubes revealed positive beta-galactosidase activity in only three organisms out of the fourteen (14) pure fungal species screened. The fungal species that tested positive for the enzyme include *Aspergillus niger* FGMTF01, *Aspergillus niger* FSMTF06 and *Geotricum candidum* FSMTF02. Table 2 summarizes the qualitative screening. Meanwhile, selected fungal isolates quantitatively screened revealed *Aspergillus niger* FGMTF01 had the highest enzymes activity of 19.60±0.34 U/ml followed by *Geotricum candidum* FSMTF02 and *Aspergillus niger* FSMTF06 with beta-galactosidase activities of 18.55±0.48 and 18.22 ±0.22 U/ml respectively. The result of the quantitative beta-galactosidase screening of the selected fungal isolates is summarized in Figure 2.

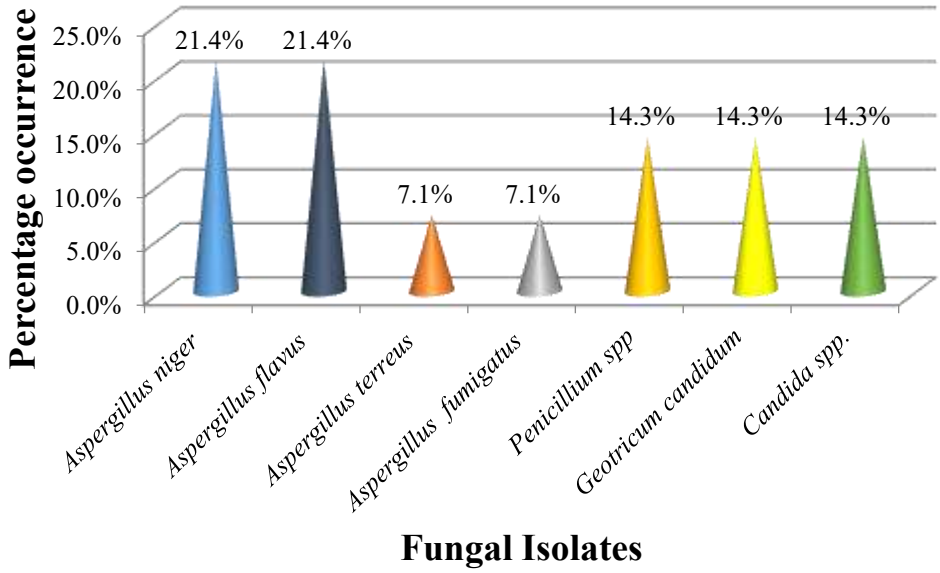

Figure 1: Frequency of Occurrence of the Fungi Isolated from Fermented Milk

Aspergillus niger FGMTF01, *Aspergillus niger* FSMTF06 and *Geotricum candidum* FSMTF02 which were positive for beta-galactosidase production from the 14 fungal isolates qualitatively screened using the YEPLB method could be attributed to the presence of the genes coding for the enzyme in these organisms. These organisms therefore may possess potentials for large scale beta-galactosidase production. On the other hand, the 11 remaining fungal species which tested negative could be attributed to the absence of the beta-galactosidase gene in these organisms or the ability of these organisms to carry out oxidative metabolism.

The findings of this investigation is in agreement with the studies of Vishwanataha *et al*. (2012), Gopalakrishnan *et al*. (2014), Silvério *et al*. (2018) who reported *Aspergillus niger* as beta-galactosidase positive organisms when screened along with different groups of fungi. It is worthy of note however, that up to the time of this report, literature is yet to be found that reports the potential ability of *Geotricum candidum* to produce beta-galactosidase. Conversely, this study disagrees with the findings of Volford *et al*. (2019), Vidya *et al*. (2020), Volford *et al*. (2021) and Bankefa *et al*. (2022) who in their separate investigations reported *Aspergillus niger* to be negative for beta-galactosidase when screened with other fungal species using different screening techniques. This disagreement could be attributed to the source of the organisms or the screening method used.

Table 2: Fungal Isolates Screened for Beta-galactosidase Production

S/No.	Fungal Isolate	Colour of YEPLB	Beta–galactosidase Activity
1	*Aspergillus niger* FGMTF01	Yellow/Orange	+
2	*Aspergillus flavus* FGMTF03	Red	-
3	*Aspergillus flavus* FGMTF04	Red	-
4	*Aspergillus niger* FGMTF05	Red	-
5	*Aspergillus terreus* FGMTF06	Red	-
6	*Aspergillus flavus* FGMTF08	Red	-
7	*Penicillium* sp. FGMTF09	Red	-
8	*Geotricum candidum* FSMTF01	Red	-
9	*Geotricum candidum* FSMTF02	Orange/Red	+
10	*Penicillium* sp. FSMTF04	Red	-
11	*Aspergillus niger* FSMTF06	Yellow/Orange	+
12	*Candida* sp. FCMTF01	Red	-
13	*Candida* sp. FCMTF02	Red	-
14	*Aspergillus fumigatus* FCMSV02	Red	-
15	Control	Red	

Keys: + (positive); - (negative); FGMTF – Fermented Goat Milk Tudun Fulani; FSMTF – Fermented Sheep Milk Tudun Fulani; FCMTF - Fermented Cow Milk Tudun Fulani; FCMSV – Fermented Sheep Milk Shanu Village

The quantitative screening of the three selected fungal isolates (*Aspergillus niger* FGMTF01, *Aspergillus niger* FSMTF06 and *Geotricum candidum* FSMTF02) showed no significant difference in the beta-galactosidase activity ($p < 0.05$). However, the highest beta-galactosidase activity found in *Aspergillus niger* FGMTF01 could be attributed to the presence of the genes and other mechanisms in the organism that up-regulate the induction of lactose substrate and subsequent expression and secretion of the enzyme. This organism therefore, could be a potential candidate for industrial scale beta-galactosidase production.

The finding of this research is in agreement with the reports of Kamran *et al*. (2017) carried out in Pakistan who reported a beta-galactosidase activity range of 25 – 42 U/mg by *Aspergillus niger*. Bankefa *et al*. (2022) in a separate study in Oye-Ekiti, South-Western Nigeria also reported *Aspergillus niger* isolated from fresh water secreted beta-galactosidase with activities between 2 to 25 U/ml. Meanwhile, the investigations of Vishwanataha *et al*. (2012), Gopalakrishnan *et al*. (2014) and Vidya *et al*. (2020) who in their separate investigations reported much higher beta-galactosidase activities of 1020IU, 120-200U/ml and 155.34±1.26 U/g respectively from strains of *Aspergillus niger* and other *Aspergillus* species which disagrees with the findings of this study. The difference in enzyme activities could be attributed to the strain and source of the fungal species as well as the methodology utilized.

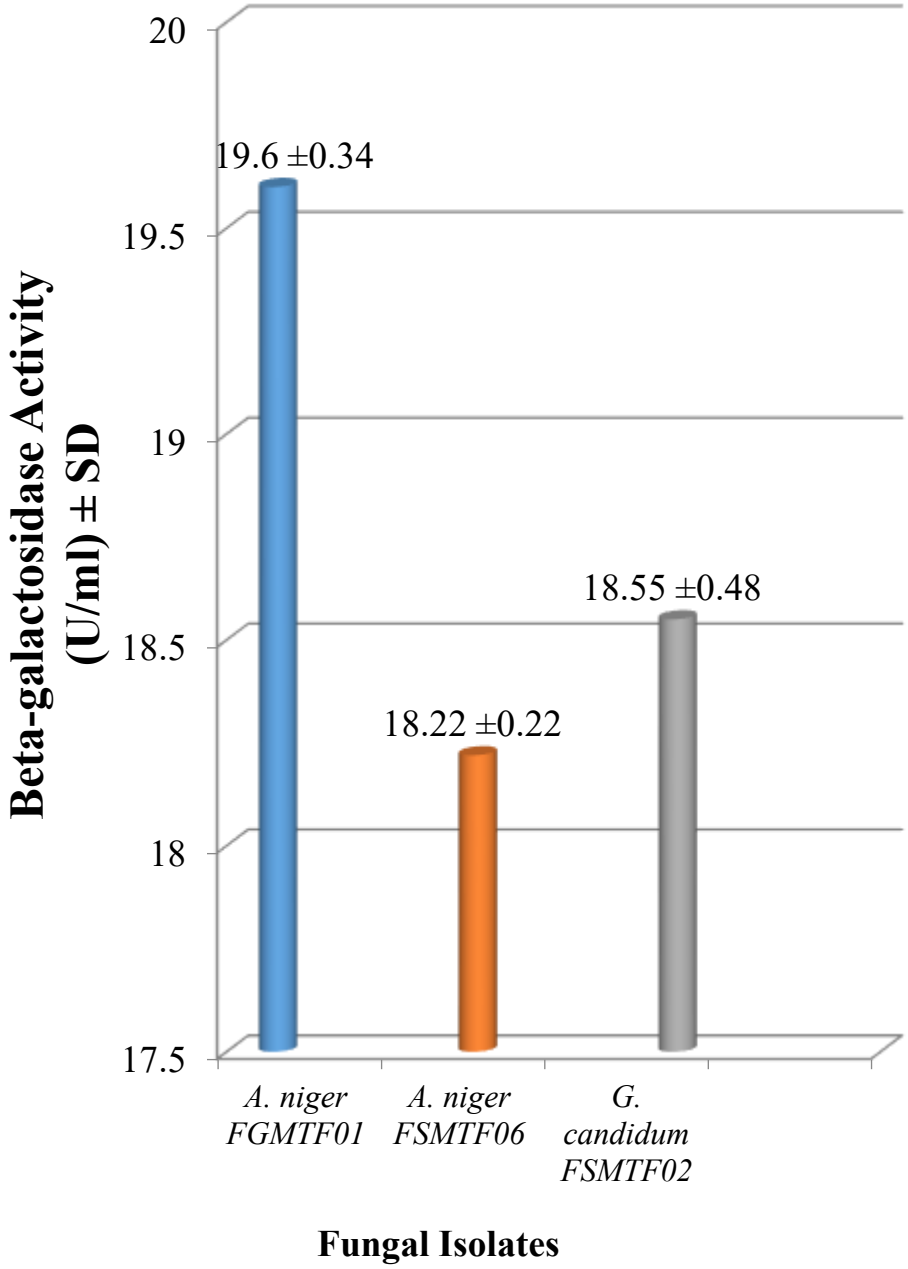

Figure 2: Fungal Isolates Screened for Beta-galactosidase Activity

Molecular Characteristics of Selected Fungal Isolates *Aspergillus niger* FGMTF01

Agarose gel image of the DNA of the selected fungal isolate, *Aspergillus niger* FGMTF01 is shown in plate PLATE XVI. A band size of approximately 600bp was

observed for the rDNA complex (ITS1 and ITS2) of the selected fungal isolate. Similar sequences of other *Aspergillus niger* isolates producing significant alignments with the *Aspergillus niger* FGMTF01 from the BLAST is summarized in Table 3. A Max score range of 89.8 to 93.5, E-value range of 3.00E-18 to 4.00E-17 and Percentage Identity range of 94.74 to 96.43% were observed. Accession numbers of the various similar *A. niger* isolates. Phylogenetic tree showing the genetic relatedness of *Aspergillus niger* FGMTF01 with other *Aspergillus niger* isolates is shown in Figure 3.

The approximate band size of 600bp observed in the electrophoretic analysis of the gene confirmed the DNA of the *Aspergillus niger* FGMTF01 as rDNA (ITS1 and ITS2 regions) of fungal origin. In addition, the DNA sequence of *Aspergillus niger* FGMTF01 was similar to various DNA sequences of *Aspergillus niger* with high max scores, percentage identities and significantly low E-values. The organism was found to be genetically related with various other species of *Aspergillus niger* isolated from India, Nigeria, Saudi Arabia, China, Iraq, Egypt and Morocco.

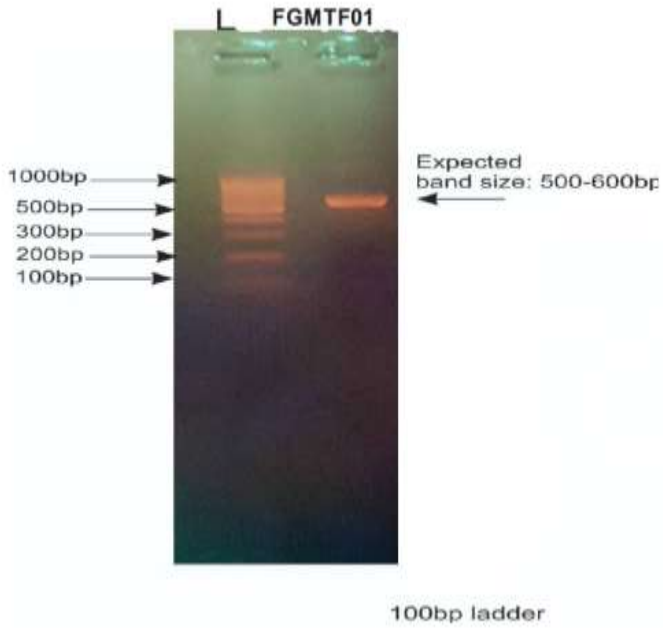

PLATE I: Agarose Gel Image of the DNA of Selected Fungal Isolate

Keys: L – Ladder; bp – base pair; FGMTF01 – *Aspergillus niger*

Table 3: BLAST showing some Strains of *Aspergillus niger* having Significant Similar Sequences with *Aspergillus niger* FGMTF01

Scientific Name	Max Score	E value	Percentage Identity	Accession	Location
Aspergillus niger	93.5	3.00E-18	96.43	KX852296.1	India
Aspergillus niger	93.5	3.00E-18	96.43	KU350621.1	Nigeria
Aspergillus niger	93.5	3.00E-18	96.43	LN832991.1	Saudi Arabia
Aspergillus niger	93.5	3.00E-18	96.43	KJ848716.1	China
Aspergillus niger	91.6	1.00E-17	94.92	OP380088.1	China
Aspergillus niger	91.6	1.00E-17	94.74	OK091635.1	Iraq
Aspergillus niger	89.8	4.00E-17	94.74	OR731146.1	Nigeria
Aspergillus niger	89.8	4.00E-17	94.74	OR668932.1	Egypt
Aspergillus niger	89.8	4.00E-17	94.74	OR668928.1	Egypt
Aspergillus niger	89.8	4.00E-17	94.74	OR651268.1	Morocco

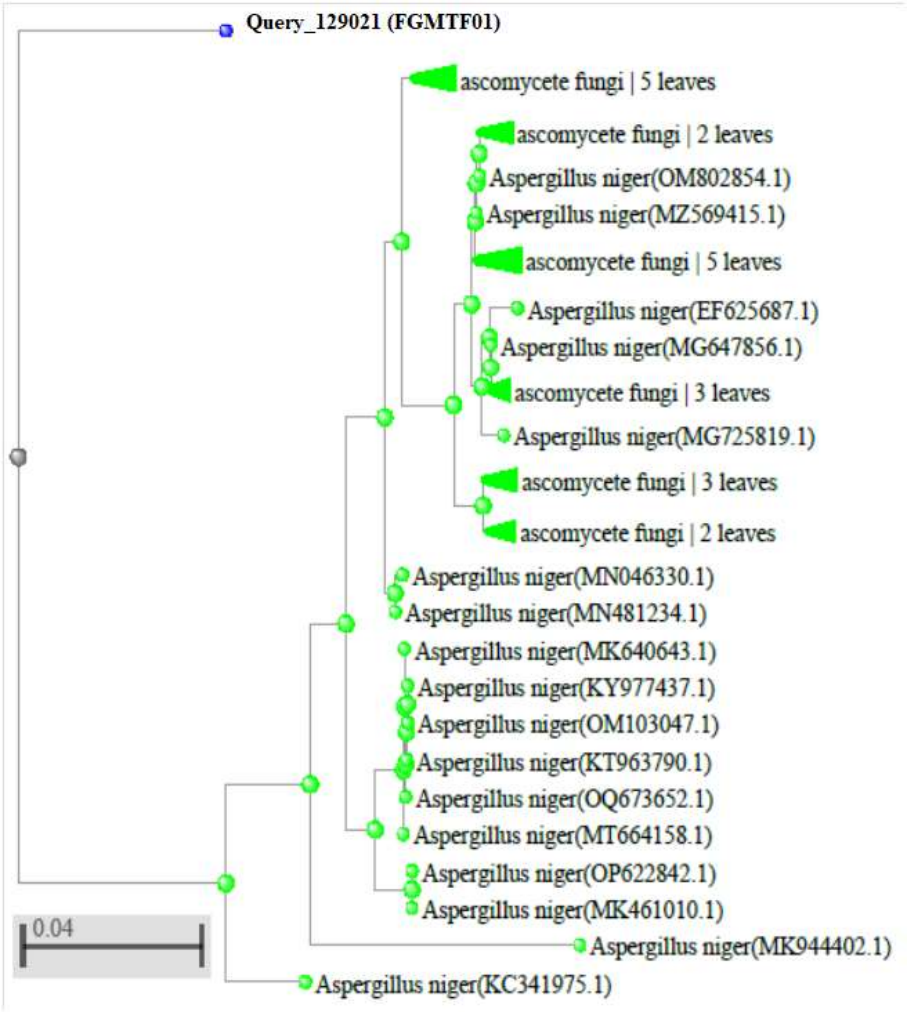

Figure 3: Phylogenetic Tree Showing Genetic Relatedness of *Aspergillus niger* FGMTF01 with other *Aspergillus niger* Isolates

Conclusion

A total of fourteen fungi were isolated and characterized from fermented cow, goat and sheep milk. The fungal isolates were identified as members of the *Aspergillus*, *Penicllium*, *Geotricum* and *Candida* Genera. Species of *Aspergillus* was the most frequently occurring fungal group isolated from the fermented milk samples. Three fungal isolates (*Aspergillus niger* FGMTF01, *Aspergillus niger* FSMTF06 and *Geotricum candidum* FSMTF02.) tested positive for beta-galactosidase production out of the fourteen isolates qualitatively screened. All the three selected fungal isolates produced beta–galactosidase with appreciable enzyme activities ranging from 18.22-19.60U/ml. The selected fungal isolate (*Aspergillus niger* FGMTF01) was confirmed molecularly with high sequence similarities and genetic relatedness with other species of *Aspergillus niger* isolated from Nigeria and other parts of the world. Additional research should be carried out to isolate fungi from other environmental sources and screen same for possible potentials of beta-galactosidase production. However, all the fungal isolates encountered in this study should be further characterized and identified using molecular techniques. The isolates should also be screened for potentials to produce other metabolites with industrial and biotechnological importance such as other enzymes beside beta-galactosidase, organic acids as well as amino acids. Fungal species that were positive for beta-galactosidase production should be further studied for large scale production of the enzyme.

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