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Millet Cobs: A Source of Microbial Enzymes

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Abstract

Microbial enzymes have significant biotechnological application in industries. The purpose of this study was to isolate and identify microorganisms associated with millet cobs, determine the enzyme activity (lipase, protease, pectinase, cellulase and amylase) of the millet cob samples, screen the isolated microorganisms for enzyme production and determine the physicochemical parameters of the degrading medium. A total of seven bacteria and twelve fungi consisting of yeasts and moulds were isolated during the study. Day 20 of the degradation period has the highest enzyme activity for all the enzymes in the degraded millet cobs; lipase has the highest enzyme activity with a value of 0.496 mg/mL/min while protease has the lowest with a value of 0.003 mg/mL/min. All of the isolated microorganisms exhibited enzymatic activity except *Zygosaccharomyces rouxii* in which *Bacillus* spp were screened positive for all the enzymes assayed for. The temperature (ºC), pH and titratable acidity (%) ranged from 24.03-28.47, 3.81-6.50 and 2.31-4.21 respectively. This study contributes to catalogue of microorganisms that has been identified as enzyme producers and provides additional information to support future research about the industrial potential of these microorganisms that may produce enzymes and other metabolites of industrial importance.

Keywords: Millet cobs; Enzymes; Bacteria; Fungi; pH, Temperature

Introduction

Many microorganisms such as bacteria, mould, and yeast produce a collection of multipurpose enzymes with extensive diversity of structures and industrial applications. Many microbial enzymes, such as amylases, cellulases, lipases, pectinases and proteases extracellularly produced. Amylases, starch-degrading enzymes, have numerous biotechnogical applications. These enzymes are used in textile and garments, paper industries, starch liquefaction, food, adhesive and sugar production and pharmaceuticals [1]. Cellulases, sugar degrading enzymes are used in textile industry for bio-polishing of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness [2]. Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juice and in baking, while de-inking of paper is yet another emerging application [3]. Lipases, lipid degrading enzyme are versatile tool for biotechnology. It is applicable in multiple industries such as agrochemical, pharmaceutical, cosmetic and perfume, taste and flavor industries, textile, food and dairy, detergent and surfactant industries, fat and oil, leather and paper production, chemical and waste water treatment [4]. Especially lipases are applied for biodiesel production [5]. Pectinases a group of enzymes that contribute to the degradation of pectin by various mechanisms. Acidic pectic enzymes are widely used in the production and clarification of fruit juices and wines [6]. They are also very important in maceration and solubilization of fruit pulps. Alkaline pectic enzymes have been used in several areas, including retting and degumming of fiber crops, textile processing, coffee and tea fermentations, paper and pulp industry, and oil extraction [7]. Proteases, enzyme which catabolizes protein by hydrolysis of peptide bonds are generally used in detergents, food industries meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds [8-10]. They also have medical pharmaceutical applications [9]. The increase in world enzyme demand has led to sourcing for alternative substrate for the production of microbial enzyme; hence, agricultural wastes are readily accessible around the world as residual wastes for the production of these enzymes. In Nigeria, the most abundant renewable biomass resources include crop residues, such as corn straw, millet cobs, cassava peels, yam peels and rice husks.

Millets are a group of highly variable small seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food. They do not form a taxonomic group, but rather a functional or agronomic one. Millets are important crops in the semi-arid tropics of Asia and Africa (especially in India and Nigeria), with 97% of millet production in developing countries and according to FAO statistics, the world production of millets was 26.7 million metric tonnes. The crop is favoured due to its productivity and short growing season under dry, high-temperature conditions [11,12]. Millet cobs are a great source of lignocellulosic biomass which is renewable, chiefly unexploited and inexpensive. Millets are a group of highly variable small seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food. They do not form a taxonomic group, but rather a functional or agronomic one. Therefore, the present research work undertaken is to determine the suitability of millet cobs for microbial enzymes production.

Materials

Collection of sample

Pearl millet (*Pennisetum glaucum*, Linn) was obtained from a farmland in Gusau, Zamfara State, Nigeria. The millet cobs were kept in a sterile air tight polythene bags and transported to the Microbiology Postgraduate Laboratory, Federal University of Technology, Akure for further analysis.

Preparation of millet cobs

The millet cobs were sun-dried for 3 weeks and grinded into powder using an electric blender (Binatone Blender). The grinded millet cobs

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were divided into two portions; A and B. Portion A was left undegraded, while Portion B was degraded for 20 days.

Liquid substrate degradation

In the submerged substrate degradation, 10.0 g of the portion B of the substrate was naturally degraded by soaking in 100.0 mL of sterile distilled water. Liquid substrate degradation was carried out during which analyses such as: microbial analysis; pH, temperature and titratable acidity determination and enzyme activity were carried out at 4 days interval during millet cobs degradation.

Isolation and identification of microorganisms

Nutrient agar (NA), nutrient broth (NB), De Man Rogosa and Sharpe Agar (MRSA), De Man Rogosa and Sharpe broth (MRSB), Potato dextrose agar (PDA), Potato dextrose broth (PDB) were prepared according to manufacturer's specification for the isolation of bacteria and fungi. Serial dilution was carried out according to the standard method of Fawole and Oso for the isolation of bacteria ($\times 10^6$ cfu/mL) and fungi ($\times 10^4$ sfu/mL) [13]. Conventional methods were used in identification of the isolated bacteria and fungi using the standard methods of Fawole and Oso [13]. Molecular identification of isolated bacteria and yeasts were carried out using the standard methods of Tamura, (2011) [14].

Determination of pH

The electrode of the meter was aseptically inserted into the media after standardization with the appropriate buffer (4 and 7 solutions). The electrode was then left in the media for 3 minutes to stabilize after which the pH was read at the same time on the meter scale.

Determination of temperature

Determination of temperature was done using a thermometer. The thermometer was inserted into the substrate 4 day interval to monitor the temperature.

Determination of total titrable acidity (TTA)

The total titratable acidity of the fermenting extrudates was determined every four day interval as described by AOAC (2012) [15]. Two grams (2 g) of the sample was weighed into 20 ml of sterile distilled water and filtered. 10 mL of the filtrate was measured and few drops of phenolphthalein indicator added. This was titrated with 0.1 M sodium hydroxide (NaOH) solution and the titre values in milliliter were added from the burette.

Enzyme activity

The enzyme activity of both the undegraded and degraded millet cobs sample was determined using the techniques for cellulase, protease, lipase, pectinase and amylase respectively [16-20].

Microbial screening for enzyme production

Culture media specific to each enzyme were used for primary screening of enzymes production by following the methods for cellulase, protease, lipase, pectinase and amylase respectively [21-25].

Statistical analyses

The experimental design was done in triplicate using complete randomization. The data obtained were subjected to analysis of variance (ANOVA) and the means were separated using Duncan's New Multiple Range Test.

Results

Microbial isolation and identification

The total bacterial counts (10^6 cfu/ml), fungal mean counts (10^4 sfu/ml) is shown in Figure 1 and 2. The total bacterial count in the undegraded sample was 7.33, while that of fungi was 8.67. The total bacterial counts for the degraded sample ranged from 28.00 to 34.33, while the range for fungi was 7.00-36.00. The conventional identification of microorganisms isolated from the millet cobs samples is shown in Tables 1-3. The frequency of occurrence of isolated microorganisms is shown in Tables 4 and 5. Molecular identification for *Bacillus cereus, Bacillus subtilis, Bacillus licheniformis*, *Corynebacterium fasciens, Lactobacillus lactis, Lactococcus lactis* and *Flavobacterium columnare* respectively which were later identified using 16S rDNA analysis to be *Bacillus licheniformis* strain ZULMMI012, *Bacillus subtilis* strain b17a, *Macrococcus carouselicus* strain H8B16, *Bacillus cereus* strain pBCO_5, *Lactobacillus brevis* strain 14.8.28, *Lactococcus lactis* subsp. lactis strain Mast_19 and *Flavobacterium ferrugineum* while that of yeasts: *Saccharomyces cerevisae, Candida albicans, Candida krusei*, *Geotrichum albidium* and *Zygosaccharomyces rouxii* respectively which were also identified using 16S rDNA analysis to be *Candida albicans* strain h70b, *Saccharomyces cerevisiae* strain K289-3A, *Rhodotorula mucilaginosa* strain G20, *Saccharomyces cerevisiae* strain BY4742 and *Kluyveromyces marxianus* strain GX-15 respectively (Figure 3a and 3b).

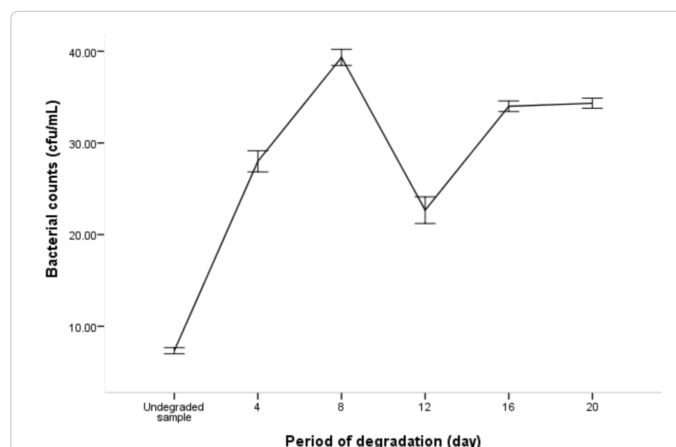

Figure 1: Bacterial counts isolated from millet cobs during degradation.

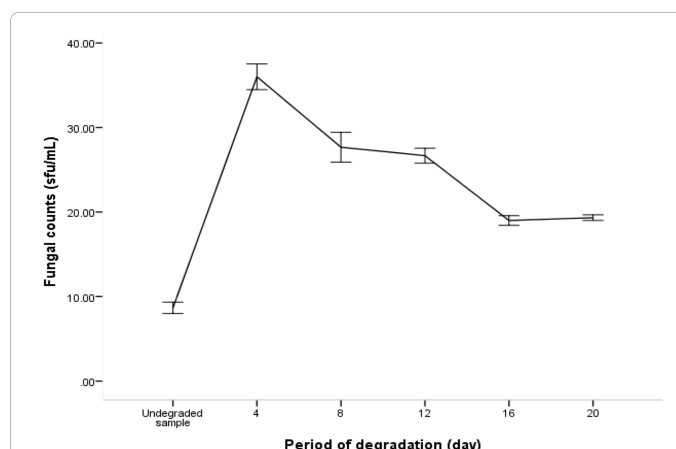

Figure 2: Fungal counts isolated from millet cobs during degradation.

Laboratory Ref. No	B1	B2	B3	B4	B5	B6	B7
Colony/Morphology							
Colour	Creamy	Creamy	Creamy	Yellow	Creamy	Creamy	Yellow
Surface	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Translucent
Cell Characteristics (microscopy)	Straight Rods	Opaque	Straight Rods	Single, straight and short rods	Straight single rod on short chain	Cocci in pairs	Singly cocci
Biochemical Test							
Gram's reaction	+ve	+ve	+ve	+ve	+ve	+ve	-ve
Catalase Test	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Motility	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Spore	+ve central	+ve central	+ve central	-ve	-ve	-ve	-ve
Indole Production	+ve	-ve	-ve	-ve	-ve	+ve	-ve
Starch hydrolysis	+ve	+ve	+ve	-ve	-ve	-ve	+ve
Citrate utilization	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Methyl red	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Sugar Fermentation							
Glucose	A-	A-	A-	AG	A-	A-	AG
Fructose	A-	A-	A-	--	AG	A-	A-
Maltose	A-	A-	A-	AG	AG	A-	AG
Lactose	--	--	--	AG	AG	A-	A-
Sucrose	A-	A-	--	AG	AG	A-	AG
Mannitol	A-	A-	--	--	--	--	A-
Probable Bacterium	*Bacillus licheniformis*	*Bacillus subtilis*	*Bacillus cereus*	*Corynebacterium fascians*	*Lactobacillus lactis*	*Lactococcus lactis*	*Flavobacterium columnare*

Key: +ve = Positive, -ve = Negative, AG = Acid and Gas, A- = Gas, -- = Acid and gas absent

Table 1: Morphological and Biochemical Characteristics of Bacterial Isolates from millet cobs.

Isolates	Cultural characteristics	Spores/conidia arrangement under the microscope	Identity of isolates
F1	Colonies are blue-green with a suede-like surface consisting of a dense felt of conidiophores	Uniserate and columnar conidial heads with the phalides limited to the upper two third of the vessicle and curving to the roughly parallel to each other	*Aspergillus fumigatus*
F2	Spores are granular, flat, often with radial grooves, yellow at first but quickly becomes bright to yellow-green with age	Conidia are globose to subglobose, pale green and conspicuously echinulate	*Aspergillus flavus*
F3	Spores are white at initial stage and turn grey with maturity	Hyphae is typically branching and form septate	*Aspergillus candidus*
F4	Conidia vary in colour from white to grey	Chains of single-celled conidia produced in basipetal succession from a specialized conidiogenous cell	*Scorpulariopsis brevicaulis*
F5	Conidia grows rapidly, resemble cotton candy and darken with age	Mycelia are marked by numerous stolons connecting groups of long sporangiophores	*Rhizopus stolonifer*
F6	Conidia appears orange	Microconidia are absent, chlamydospores are intercalary, exceptionally terminal, spherical to ovoidal	*Fusarium poae*

Table 2: Morphological Characteristics of Mould isolates from biodegraded millet cobs.

Laboratory Ref. No	Cell shape	Morphology					Biochemical Properties Fermentation/Assimilation						Yeast Identity
		Ascospore											
		Present/Absent	Shape	Spore	Pseudomycelium	Mycelium	Glucose	Fructose	Sucrose	Lactose	Maltose	Nitrate	
Y_1	Oval	+	Oval	+	+	-	FA	FA	FA	-A	FA	-	*Sacharomyces cerevisae*
Y_2	Cylindrical	+	Oval	+	+	-	FA	FA	FA	-	FA	+	*Candida albicans*
Y_3	Cylindrical	+	Ovoid	+	+	-	FA	FA	FA	FA	FA	-	*Candida krusei*
Y_4	Cylindrical	+	Short Cylindrical	+	-	+	FA	FA	-A	-A	FA	+	*Geotrichum albidium*
Y_5	Spherical	+	Spherical	+	+	-	FA	FA	FA	-A	FA	-	*Zygosaccharomyces rouxii*

Key: + = Present, - = Absent, FA= Fermentation and Assimilation, -A= Assimilation only

Table 3: Morphological and Biochemical Characteristics of Yeasts isolated from millet cobs samples.

Bacterial isolates	Degradation Period (day)						Occurrence (%)
	Undegraded sample	4	8	12	16	20	
Bacillus cereus	+	+	+	-	-	-	17.79
Bacillus licheniformis	+	+	-	-	-	-	14.65
Bacillus subtilis	+	+	+	-	-	-	16.76
Corynebacterium fasciens	-	-	-	+	+	+	12.77
Flavobacterium ferrugineum	-	+	+	+	+	-	9.79
Lactobacillus lactis	-	+	+	+	+	+	16.10
Lactococcus lactis	-	+	+	+	+	+	12.14
Mould isolates	Degradation Period (day)						Occurrence (%)
	Undegraded sample	4	8	12	16	20	
Aspergillus flavus	+	+	+	-	-	-	16.41
Aspergillus fumigatus	+	+	-	-	-	-	16.37
Aspergillus candidus	-	+	+	+	+	-	20.39
Fusarium poae	-	-	-	+	+	+	17.03
Rhizopus stolonifer	+	+	+	+	-	-	17.07
Scopulariopsis brevicaulis	-	-	-	+	+	+	12.73
Yeast isolates	Degradation Period (day)						Occurrence (%)
	Undegraded sample	4	8	12	16	20	
Candida albicans	+	+	-	-	+	+	25.76
Candida krusei	+	+	-	-	-	-	19.18
Geotrichum albidum	-	-		+	+	+	20.83
Saccharomyces cerevisiae	-	-	+	+	+	+	23.90
Zygosaccharomyces rouxii	-	+	-	-	-	-	10.33

Table 4: Frequency of occurrence of microbial isolates from millet cobs during degradation.

Conventional identification of isolates	Sequence with best match Accession no	Sequence based with Similar strain	Base Pair	Similarity (%)
Bacillus cereus	MH411110.1	*Bacillus licheniformis* strain ZULMMI012	1260	100
Bacillus subtilis	SPP54602	*Bacillus subtilis* strain b17a	1453	95
Bacillus licheniformis	NR_044927.1	*Macrococcus carouselicus* strain H8B16	1549	85
Corynebacterium fasciens	CP009965.1	*Bacillus cereus* strain pBCO_5	4983	100
Lactobacillus lactis	KX301062.1	*Lactobacillus brevis* strain 14.8.28	826	94
Lactococcus lactis	JQ953678.1	*Lactococcus lactis* subsp. lactis strain Mast_19	678	99
Flavobacteriun columnare	AF335328.1	*Flavobacteriun ferrugineum*	589	100
Saccharomyces cerevisiae	KP674770.1	*Candida albicans* strain h70b	510	80
Candida albicans	AF058447.1	*Saccharomyces cerevisiae* strain K289-3A	1227	97
Candida krusei	KY296083.1	*Rhodotorula mucilaginosa* strain G20	613	97
Geotrichum albidium	NM_001181492.1	*Saccharomyces cerevisiae* strain S288C	1227	99
Zygosaccharomyces rouxii	FJ896138.1	*Kluyveromyces marxianus* strain GX-15	556	99

Table 5: Conventional and Molecular Identification of microorganism isolated from Millet Cobs during degradation.

Temperature and pH variation of the millet cob samples

Figures 4 and 5 shows the temperature (°C) and pH variation of both the undegraded and degraded millet cob samples respectively. The highest temperature value was recorded on day 16 with value 28.47 ± 0.03 while the lowest temperature was recorded on day 4 with a value of 24.03. The control sample has the highest pH with a pH of 6.50 while the lowest pH was recorded on day 20 of the biodegradation period with a pH of 3.81.

Titratable acidity of the millet cob samples

Figure 6 show that the undegraded sample (2.31) has the lowest titratable acidity (%), while the highest was on day 8 (4.21). There were no significant difference (P>0.05) between the values of days; 12 (4.00), 16 (4.05) and 20 (4.07), however, there was significant difference (P<0.05) between these days of degradation and day 8.

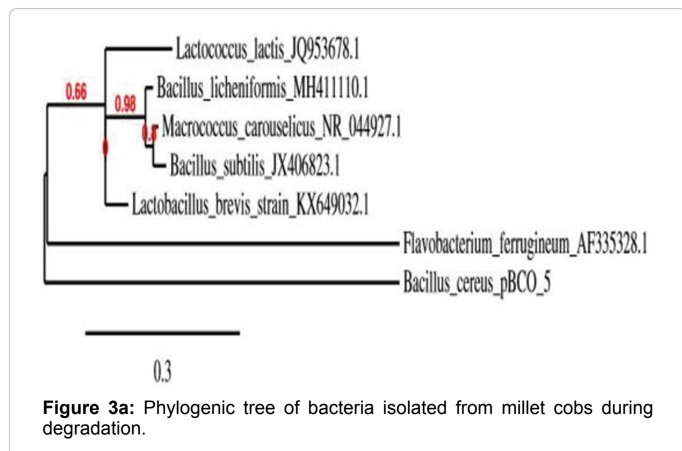

Figure 3a: Phylogenic tree of bacteria isolated from millet cobs during degradation.

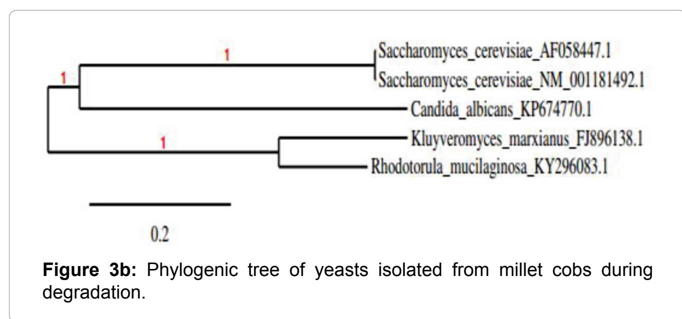

Figure 3b: Phylogenic tree of yeasts isolated from millet cobs during degradation.

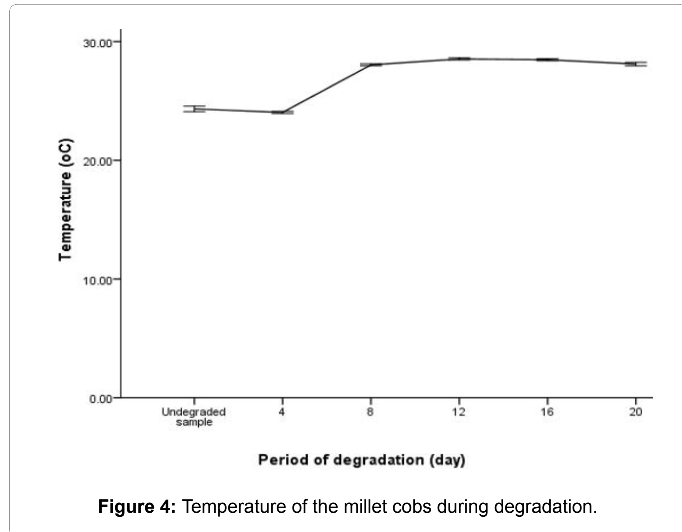

Figure 4: Temperature of the millet cobs during degradation.

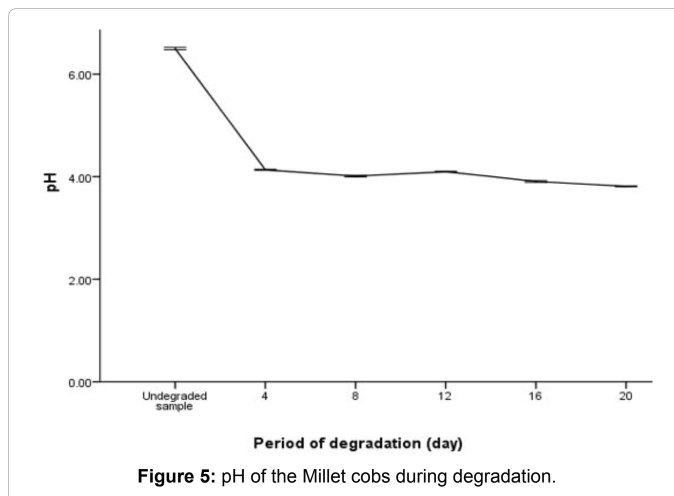

Figure 5: pH of the Millet cobs during degradation.

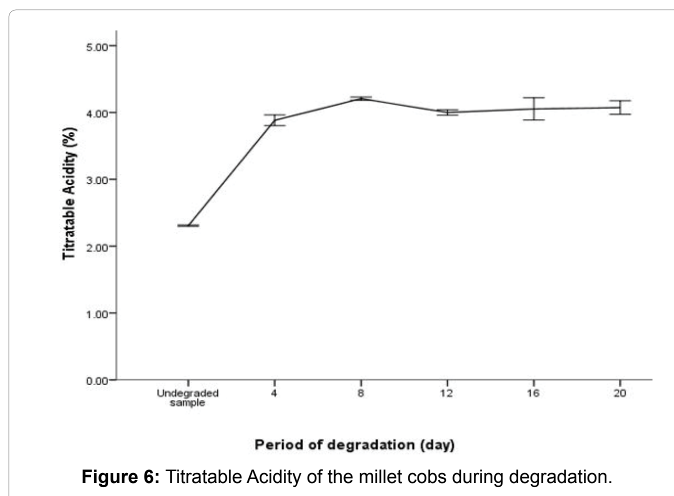

Figure 6: Titratable Acidity of the millet cobs during degradation.

Bacteria	Pectinase	Lipase	Cellulase	Amylase	Protease
B. cereus	+	+	+	+	+
B. licheniformis	+	+	+	+	+
B. subtilis	+	+	+	+	+
Corynebacterium fascians	-	+	+	+	+
Flavobacterium ferrugunieum	-	-	-	-	+
Lactobacillus lactis	-	+	+	+	+
Lactococcus lactis	-	+	+	+	+
Fungi	**Pectinase**	**Lipase**	**Cellulase**	**Amylase**	**Protease**
Saccharomyces cerevisae	+	+	+	+	+
Geotrichum albidum	+	-	-	-	-
Aspergillus fumigatus	+	-	+	+	+
Aspergillus flavus	+	-	+	+	+
Candida krusei	+	-	+	-	+
Aspergillus candidus	+	-	+	+	-
Scorpulariopsis brevicaulis	+	-	+	-	-
Rhizopus stonifer	+	-	+	+	+
Candida albicans	+	+	-	+	+
Fusarium poae	+	-	-	-	+
Zygosaccharomyces rouxii	-	-	-	-	-

Table 6: Microbial screening for enzyme production.

Enzyme activity and microbial screening for enzyme production

The enzyme activities of the millet cob a sample is shown in Figure 7. Lipase has the highest enzyme activity which was recorded on day 20 of the degradation period. The enzyme activities for all the enzymes assayed for were low in the control sample. All the isolated microorganisms exhibited enzymatic activity except *Z. rouxii* (Table 6).

Discussion

Variation observed in names given using conventional and molecular methods of identification of microorganisms agrees with the findings of Frickmann [26]. They reported differences in names

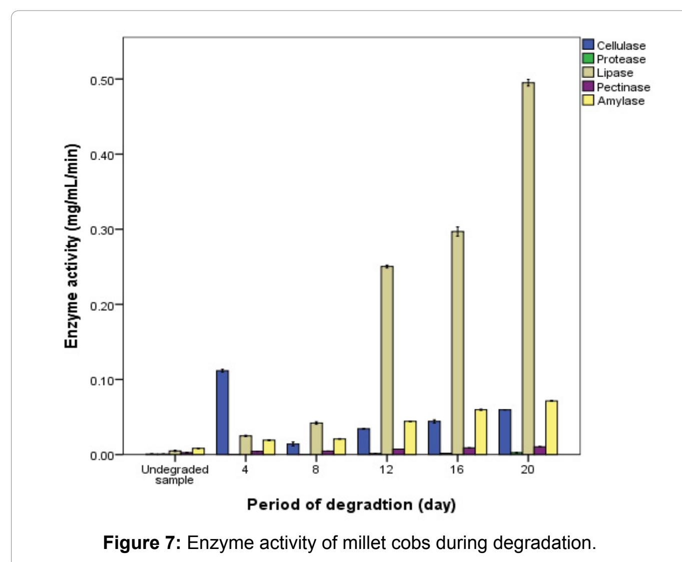

Figure 7: Enzyme activity of millet cobs during degradation.

allotted using conventional and molecular methods of identification. However, the results of this study demonstrated clearly the interest and feasibility to introduce the 16SrRNA gene sequencing method in identification of bacteria and yeasts. Combination of conventional techniques and molecular approach will improve microbiological study and verification, allowing for exclusive and effective identification of microorganisms as against using only conventional method of identification.

The lignocellulosic structure of millet cobs maybe a factor responsible for the low microbial population present particularly in the undegraded sample [27-29]. The presence of bacteria such as *B. cereus*, *B. licheniformis* and *B. subtilis* in the undegraded sample agrees with the findings of Kunchala when sorghum and pearl millet from semi-arid tropics were characterized for potential probiotic bacteria [30]. The presence moulds such as A. fumigatus, *A. flavus* and *R. stolonifer*, in the undegraded sample could be a result of them being millet cobs microflora which agrees with the finding of Badau when *A. nidulans*, *A. niger*, *R. arrhizus* were isolated from unmalted millet grains. Bamigboye also isolated *A. flavus*, *A. niger*, *Rhizopus oryzae* from corn cobs [31,32]. Slightly acidic environment of the millet cobs may be responsible for their adaptation [33,34]. The dominant population of *A. fumigatus*, *A. flavus* particularly in the undegraded sample maybe due to the presence of nutrients available within the millet cobs for utilization. Nasrin attested to this when combination of molasses and jackfruit were used as a substrate for mutant strain of *A. niger* for citric acid production [34]. The high counts of bacteria, mould and yeast in the millet cobs during degradation may be due to the high concentration of nutrients which agrees with the findings of Arotupin that arrays of microorganisms could increase in population due to the nutrient rich nature of the substrate, thus, supporting the growth and proliferation of microorganisms [35]. Low pH could be responsible for the increased fungal population [36].

Lactobacillus lactis and *Lactococcus lactis* are vital organisms for fermentation process which may be responsible for their presence from during degradation. Kalui documented in a review that these bacteria are essential in spontaneously fermenting cereal based food and that these microorganisms produce lactic acid as an important product from the energy yielding fermentation of sugars [37]. Ogbonnaya and Chukwu also attested to this; that majority of lactic acid bacteria isolated from 'Akamu' which is made from maize, a cereal belonged to the Genera: *Lactobacillus* [38]. Hence, these microorganisms may be implicated as partly responsible for initiating acidification during degradation of millet cobs. *Saccharomyces cerevisae* and *Aspergillus* spp may as well be responsible for lactic acid production which concurs with the documentation of Kalui that some yeasts (*Saccharomyces*) and moulds (*Penicillium*, *Aspergillus* and *Botrytis*) too produce lactic acid [37].

Lactococcus spp also produce ammonia from arginine [37]. All these potential bye-product of *L. lactis* may be responsible for the reduction of other bacterial population which was obtained in the undegraded sample. These conditions created by *Lactobacillus lactis* and *Lactococcus lactis* could favour the growth of fungi particularly on day 4 and beyond, thus, Fungi, *Lactobacillus* and *Lactococcus* metabolise sugars within the millet cobs which is converted to organic acids.

The growth and population of other microorganisms maybe due to the creation of enabling environment which favour their survival, growth and development of these set of microorganisms. Lactobacilli lactis and *Lactococcus latis* and mould had the highest population counts towards the end of the degradation period. These conform to the report of Ogbonnaya and Chukwu [38]. *Saccharomyces* spp, a cabtree positive yeast could utilize the sugars present within the millet cobs for the facilitation of the fermentation process in order to accumulate ethanol in the presence of oxygen particularly during early days of degradation period [39-41]. *Candida albicans* disappearing on day 8 may be due to the decrease in the available sugars within the millet cobs which might have facilitated their survival, growth, development and proliferation, however, *S. cerevisiae* becoming dominant on day 12 may be due to the environmental conditions that facilitate their survival, growth, development and proliferation. *Candida* spp, a cabtree negative yeast catabolizes sugars into carbon dioxide in the presence of oxygen may create an enabling environmental condition which may have facilitated the occurrence of *S. cerevisae* during degradation [42]. However, the reduction in the numbers of *S. cerevisiae* on days 16 and 20 maybe due to their preference for sugars [42]. Increase in the number of *Candida* spp over *Saccharomyces* spp on day 16 and 20 maybe due to their utilization of sugars. The reduction in yeast population of days 16 and 20 as compared to day 4 may be due to reduced oxygen [39-41].

The increased population of *A. flavus*, *A. fumigatus* and *R. stolonifer* in day 4 may be as a result of the reduced lignocellulosic component of the millet cobs [27-29]. The increased moisture content of the degraded millet cob as the degradation progressed could be responsible for the reduction in the fungal population [43]. The growth of *A. Candidus* and *F. poae* on days 8 and 12 respectively succeeding *A. fumigatus* maybe a result of factors that may not favour its growth such as low relative humidity [44]. The availability of light may be responsible for the growth of *A. fumigatus* and *A. flavus* particularly in the undegraded sample, degraded sample of day 4 which also agrees with the findings of Shehu and Bello while studying the effect of environmental factors on the growth of *Aspergillus* spp associated with stored millet grains [44]. The growth of *A. flavus*, though in small population on day 8 shows that *A. flavus* has a higher tolerance level than *A. fumigatus* in darkness. The presence of *A. candidus* from day 8 to day 20 with a relative progressive rise in population could be as a result of increase in the dark condition as the degradation process progresses [44]. The occurrence of *R. stolonifer* on day 8 to day 12 could be as a result of these factors (ability to grow under low relative humidity and in the dark). *Scopulariopsis brevicaulis* occurred on day 12 and day 20 in which it was the only mould that grew on day 20 of the degraded millet cobs. Hence, *S. brevicaulis* have the highest survival rate of growing in the dark and in low relative humidity. Also, as the biodegradation

period increases, relative humidity and light intensity decreases. Thus, these factors could be applicable to bacteria and yeast that grew on the degraded millet cobs.

Millet cobs could serve as a fermentation media for the production of enzymes. Adeleke documented that the production of enzymes by microorganisms in the fermentation media depend on the availability of suitable and utilizable substrate [45]. Rashid concurred that major and minor elements contained within a substrate can be degraded or synthesized by microorganisms using various enzymes [43]. Also, the capacity of microorganisms to produce extracellular enzymes is influenced by environmental conditions such as temperature, pH, aeration, inoculums age and the presence of inducer or repressor substrates [46,47].

The low amount of enzyme activity of all the enzymes assayed for particularly in the undegraded sample may be due to complex structure such as cellulose, hemicellulose and lignin which is important for its utilization and digestibility [33,48-50]. Solid state fermentation for the production of enzymes offers advantages over the conventional method of submerged fermentation [51]. Submerged method of fermentation used may also be attributed to the low enzyme activities [52]. The progressive increase in enzyme activities may be due to the hydrolytic (water) effect on the millet cobs which may increase the surface area and remove hemicellulose. This is in agreement with the findings of Rodolfo that water treatments at elevated temperatures (200-230°C) and pressures can increase the biomass surface area and remove hemicellulose [53]. The enzyme activity of the millet cobs may have increased if it temperature and pressure were raised.

The presence of cellulase in millet cobs agrees with the separate reports of Sethi and Philip which states that enormous amounts of agricultural, industrial and municipal cellulose wastes contains cellulose [54,55]. The increased cellulase activity on day 4 may be due to the low amount of sugars recorded particularly glucose which may inhibit the production of cellulase while the low cellulase activity on day 12 and the undegraded sample may be due to the increased glucose composition inherent within the millet cobs. It could also be due to the reduced amount of disaccharide cellobiose which could be present within fermented millet cobs which seems to be a more potent inhibitor of cellulase [56]. Payne et al. documented that a large number of compounds such as glucose, mannose, galactose, xylose, ethanol and various ions can act as possible inhibitor for cellulose [57]. The undegraded sample offered reduced accessibility to cellulose and hemicellulose and degradability for enzymatic or chemical action which agrees with the findings of Barakat and Gao [56,58].

The presence of *Bacillus* spp may contribute to the production of cellulase in the degrading millet cobs which were shown by the zone of hydrolysis in their screening for cellulase production. Separate findings of Akhtar and Saowapar agrees with this finding when different species of *Bacillus* produced cellulose [21,29]. Shilpa and Pethe also isolated different cellulolytic bacteria from the soil in which *B. thurigenesis* showed the highest zone of hydrolysis when these bacteria were optimized at different condition [59]. The pH may also be a limiting factor to the low production of cellulase within the millet cobs during degradation. Shilpa and Pethe documented that *Bacillus subsp subtilis* A-54 has optimum pH of 6.5 and stable in pH range of 6.5-8.0 [59]. Sadhu and Maiti stated that the optimum temperature for *Bacillus* sp to produce cellulase in high quantity is between 37°C to 55°C [60]. *Aspergillus fumigatus*, *A. flavus* and *A. candidus* that were positively screened for cellulase production agrees with the separate findings of Jabasingh, Liu, Amorea and Faracoa that *A. acculeatus*, *A. fumigatus*, *A. niger* were confirmed to be producers of cellulose [61-63]. Yeasts such as *S. cerevisae* and *Candida* spp have shown capacity to produce cellulase [64].

Lactococcus lactis and *Lactobacillus lactis* being protease producers agrees with the finding of Hnin that proteolytic activity is an important characteristic of lactic acid bacteria [65]. *Bacillus* spp production of protease agrees with the findings of Hamza and Woldesenbet when *Bacillus* sp. Cab44 was observed to hydrolyse casein [65]. Alemu reported that *B. licheniformis, B. firmus, B. alcalo B. subtilis* and *B. thuringiensis* were producers of protease [66]. All the *Aspergillus* spp being producer of protease agrees with the report of Alemu that *A. flavus, A. miller, A. niger* and *P. griseofulvinare* were protease producers [66]. Oyeleke documented that *A. flavus* and *A. fumigatus* were able to produce extracellular protease [67]. Oyeleke observed that when *A. flavus* and *A. fumigatus* were subjected to the same temperature of 30°C, *A. flavus* was able to produce the highest amount of protease. *Rhizopus stolonifer*, a fungus also screened positive for protease production agrees with the findings of Devi that moulds of genera *Aspergillus, Penicillium* and *Rhizopus* are useful for producing proteases, as several species of these genera are generally regarded as safe [68]. Sharp decrease of protease activity from day 12 to day 16 could be due to the ability of microorganisms to utilize sugar faster than protein. Dash et al. reported that *Bacillus* sp isolated from soil has maximum protease activity at optimum pH of 9.0. *Bacillus amyloliquefaciens, B. subtilis, B. licheniformis* and *B. stearothermophilus* were reported to produce protease at 37-60°C [68]. Optimum temperature for protease production in *Bacillus* spp has also been reported at 35-80°C [69]. Oyeleke documented that the optimum temperature for proteases production for both *A. flavus* and *A. fumigatus* was 30°C [67]. Oyeleke documented that optimum pH for *A. flavus* is 8.0 while *A. fumigatus* is 5.0. Devi reported optimum pH of 8.5 for protease production by *A. niger*, although, this is at variance with Siala et al. that reported optimum temperature of 60 and 90°C and optimum pH of 3.0 and 9.0 for proteases produced by *A. niger* [70,71]. These factors could be responsible for protease showing low activity.

The various *Bacillus* spp isolated from the millet cob samples are the best producer of lipase based on the zone of hydrolysis. *Bacillus licheniformis* strains, *B. licheniformis* -Ht7 have been identified to be good producer of lipase [72]. Lactic acid bacteria isolated from the millet cobs during degradation exhibiting lipase activity concurred with the documentation that *Lactobacillus* sp is a producer of lipase which is used for meat degradation [73]. *Candida albicans* and *A. flavus*, screened positive for lipase production agrees with the documentation of Singh that *C. Antarctica*, *C. lipolytica* and *A. flavus* are producers of lipase [74]. Singh et al. also observed that different lipase producing bacteria and fungi produced maximum lipase between pH 5.0 and 10.0 [74]. Padmapriya et al. observed lipase production increased with increase in temperature from 30 to 40°C and maximum production of lipase was obtained at 40°C and production declined at 50°C with different *Bacillus* spp and *Lactobacillus* spp [73]. Microorganisms were not subjected to these conditions, yet, lipase activity was highest among the enzymes evaluated.

Fungi are better producers of pectinase when compared to bacteria based on the findings of this research. Only *Bacillus* spp isolated where indicated to be a producer of pectinase. This agrees with different documentations of Bayoumi et al., Geetha et al., Mukesh et al., Darah et al., Raju and Divakar, Roosdiana et al., Kavuthodi et al. and Reddy et al. that most of the Bacterial isolates (mostly *Bacillus* spp and *Pseudomonas* spp) such as; *Pseudomonas* fluorescence and *B. subtilis*, *Bacillus* sp.

MFW7, *B. cereus, B. licheniformis, B. cereus, Bacillus* sp. MBRL576, *B. firmus* I-4071, Enterobacter aerogenes NBO2, Pseudoalteromonas haloplanktis strain ANT/505, *Paenibacillus xylanolyticus, B. firmus, B. firmus* (P1), *B. coagulans* (P13), *B. endophyticus* (P57) and *B. vietnamensis* (P58), *B. subtilis* BKDS1, *Enterobacter* sp. PSTB-1 and *Staphylococcus aureus* were reported as good pectinase producers [75-82]. *Bacillus subtilis* being a producer of pectinase agrees with the report of Mariam and Aruna that *B. subtilis* strain arium 1115 produced the highest quantity of extracellular pectinase out of the arrays of *Bacillus* spp assayed for [83]. Many strains of *Bacillus* have been previously reported to produce extracellular pectinases, such as *B. subtilis* Strain NVFO 19 (El-sayed, 2015), marine *B. subtilis*, *B. subtilis* BKDS1, *B. sphaericus* MTCC 7542, *B.* sp. MBRL576, *B. licheniformis* KIBGEIB21, *B. cereus, B. subtilis, B. stearothermophilus, B. firmus* and *B. cereus* [80-89]. pH could also be a factor for low pectinase production in which acidic pH values were observed during the degradation of the millet cobs. According to the finding of Mariam and Aruna, *B. subtilis* strain arium 1115 produced the highest amount of pectinase at pH 9 (21.44 U/mL) as against pH 3 (0.61 U/mL) [83]. This could be a limiting factor for pectinase production especially for *Bacillus* spp isolated from the millet cobs sample but some species like *Bacillus* sp. MBRL576 and *B. circulans* have optimum pH at 4.0 and 7.0 respectively [81,88]. Temperature is another factor to consider for pectinase production. *Bacillus* species have demonstrated slightly higher temperature for pectinase production such as marine *B. subtilis* and *B. circulans* at 40°C [86,81]. However, findings show *B. cereus, B. firmus* I-10104, *B. cereus* and *B. endophyticus* and *B. coagulans* exhibited maximum pectinase production at 37°C [90-92]. Simran and Vijay attested that *Bacillus* subtilis isolated from different agro-industrial wastes were able to produce pectinase at a temperature of 35°C and at pH 7.0 [93]. The temperature range of millet cobs during degradation fall short of these values.

All the fungal isolates that were screened positive for the production of pectinase may be responsible for the progressive increase in pectinase activity. Arotupin et al. revealed that *A. repens* is capable of hydrolyzing pectin. The hydrolysis of pectin by *A. flavus, A. versicolor* and *A. niger* have been reported [94,95]. Arotupin et al. revealed that temperature at 30°C is optimum for the production of pectinase by *A. repens* [94].

Amylase activity in the millet cobs during degradation may be as a result of the presence of high concentration of hemicellulose carbohydrate particular starch within the millet cobs. This agrees with the findings of Singh et al. when amylase and xylanase content of rice bran, corn cob, wheat bran, wheat straw, and sugarcane bagasse were described in relation with the composition of starch and hemicellulose [95]. The progressive increase in amylase activity in the fermented millet cobs from day 4 to day 20 could be due to the availability of microorganisms such as *B. cereus, B. licheniformis, B. subtilis, Lactobacillus lactis, Lactococcus lactis, A. flavus, A. fumigatus, A. candidus, Fusarium poae, Scorpulariopsis brevicaulis, C. albicans*, and *S. cerevisae* in the degrading substrate. This consortium of microorganisms may contribute to the high amylase production. Akpomie et al. reported that *Bacillus* spp and *Lactobacillus* sp have ability to produce amylase [25]. Prakash and Jaiswal reported that *B. subtilis, B. stearothermophilus, B. lecheniformis* and *B. amyloliquefaciens* are known to be the good producers of thermostable α- amylase [96]. Sundarapandiyan and Jayalakshmi isolated, characterized and screened *Bacillus* subtilis SJ33 from marine environment for amylase production [97]. It is also to be known Prakash and Jaiswal stated in one of their findings that thermophilic bacterium *B. stearothermophilus* offers an alternative for commercial production of thermostable α-amylases [96]. Hence, *Bacillus* spp are known to be commercial producer of amylase. Filamentous fungi, such as *A. oryzae* and *A. niger* produce considerable quantities of enzymes that are used extensively in the industry [98]. Progressive increase in temperature could also be another factor that led to the progressive increase in amylase production which may be a satisfactory condition that made the microorganisms to produce more amylase. Akpomie et al. attested to this fact that gradual increase in amylase activity was observed from 26 to 45°C and beyond this range it declined [25]. Thus, temperature contributes to the factor responsible for the secretion of amylase. Cavalheiro et al. documented that high enzymatic activity for amylase production by Gongronella butleri was between pH ranges of 4.0-5.5 [99]. Nwagu and Okolo reported that amylase produced by *A. fumigatus* maintained 94% of its activity at pH 4.5-6.5 for 24 hours [99]. de-Souza and Magalhães reported that *A. niger* has important hydrolytic capacities in amylase production and its tolerance of acidity (pH<3) could be a factor in the production of amylase by different *Aspergillus* spp isolated [100]. Sujeeta et al. reported that amylase activity of yeast isolates may increase at a temperature of 30°C. The temperatures obtained during the degradation of the millet cobs were below this [101].

Conclusion

A Millet cob shows amylase, cellulytic, pectinolytic, lipolytic, proteolytic and amylytic activities when degraded by inherent microorganisms. Further studies on the millet cobs particularly on improving the microbial enzymatic activities in order to explore their potent value in commercial application should be carried out.

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