



## Isolation of Bacteria with Potential of Producing Extracellular Enzymes (Amylase, Cellulase and Protease) from Soil Samples

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors SAF and AAO designed and supervised the research. Authors OFO and AF carried out the research. Author SAF wrote the manuscript. All the authors participated in the review of the manuscript and approve it.*

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### **ABSTRACT**

**Introduction:** Amylase, cellulase and protease are known for hydrolyzing starch, cellulose and protein respectively and these enzymes can be produced by microorganisms. A single bacterium with potential of producing amylase, cellulase and protease will be an organism of high industrial value.

**Aims:** This work aimed at isolating bacteria that will be able to produce three extracellular enzymes (amylase, cellulase and protease).

**Methodology:** Soil samples were collected from eight different locations within Ajayi Crowther University, Oyo Town, Nigeria. Bacteria were isolated from these soil samples and were identified using morphological and biochemical characteristics. Isolated bacteria were screened for their ability to produce amylase, cellulase and protease on plate and enzymes' relative activities on plate were determined.

**Results:** Forty two bacteria were isolated from soil samples and identified to belong to the Genera *Bacillus* (30), *Enterobacter* (6), *Klebsiella* (3) and *Staphylococcus* (3). Eight (19%), Eleven (26%)

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and Nineteen (45%) out of 42 isolated bacteria were able to produce amylase, cellulase and protease on plate with relative activities ranging from 1.25 – 2.88, 1.39 – 4.50 and 1.13 – 5.17 respectively. All the eight amylolytic isolates (*Bacillus* species (5) and *Enterobacter* species (3)) were able to produce the three enzymes (amylase, cellulase and protease).

**Conclusion:** Eight bacteria with ability to produce three enzymes (amylase, cellulase and protease) were isolated from soil samples and could be further employed in enzyme-producing industries.

**Keywords:** Amylase; cellulose; protease; extracellular enzymes; soil samples.

## 1. INTRODUCTION

Soil harbours many microorganisms with better advantages over other sources for industrial use [1]. Among bacteria often isolated from soil are *Bacillus* species, *Pseudomonas* species, *Enterobacter* species, *Staphylococcus* species, *Klebsiella* species and others [2-4]. Amylase breaks down starch to simple sugar while cellulose is hydrolysed by cellulase and protease degrades protein [5]. Amylases are used in biofuel industries to break starch to fermentable sugar [5,6]. Amylases are used in the production of fruit juices and cakes, in brewing and baking and other processed foods [6].

Cellulases are used in textile industry and are usually added to washing powder and detergents in laundry [7]. They are used to release antioxidant from food in food industry [7]. Cellulases aid in conversion of cellulolytic materials to simple sugar during ethanol production from lignocellulose [8,9]. They play vital roles in wine industry like improvement in filtration rate and wine stability [7].

Protease used in food industry could hydrolyse proteins thereby improving the organoleptic properties of the food and could also reduce food protein allergy [10]. Proteases regulate diverse metabolic processes and are used to develop effective therapeutic agents in pharmaceutical industry [11]. They are used in the conversion of wastes to feeds and foods in waste management and are relevant and popularly used in leather industry [11]. Ability of a bacterium isolated from soil samples to produce these extracellular enzymes (amylase, cellulase and protease) will make the organism relevant and important in enzymes-producing industries. This study was designed to isolate bacteria that are able to produce amylase, cellulase and protease together.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection, Isolation and Identification of Bacteria

Soil samples deposited with waste materials were collected from eight different locations in Ajayi Crowther University, Oyo Town, Nigeria (7.8430° N, 3.9364° E). The samples were diluted and appropriate dilution was inoculated into Nutrient agar using pour plate technique. They were incubated at 37°C for 24 to 48 hours. Distinct colonies from plates were streaked out on Nutrient agar until pure cultures were obtained. Isolated bacteria were stored in Nutrient agar slant for further use. The isolates were identified using their morphological and biochemical characteristics with reference to Bergey Manual of Systematic Bacteriology [12].

### 2.2 Extracellular Enzymes Screening

Amylolytic property of the isolated bacteria was screened using Nutrient agar supplemented with 1% starch. Clear zone around the organism after flooding with Gram's iodine indicates the ability of the bacterium to produce amylase [5].

Nutrient agar supplemented with 1% carboxy methyl cellulose (CMC) was used to screen ability of the isolated bacteria for cellulase production. Appearance of a clear zone against red colour of Congo red shows that the organism has ability to produce cellulase [5].

Proteolytic ability of isolates were screened using nutrient agar supplemented with 1% skimmed milk. A clear halo along the line of streak indicates ability of the bacterium to produce protease.

Relative activities of enzymes were determined using the method of Adesina and Onilude [13].

$$\text{Relative activities} = \frac{\text{Diameter of halo on plate}}{\text{Diameter of the bacterium on plate}}$$

### 3. RESULTS

A total of 42 bacteria were isolated from soil samples collected from eight different locations within Ajayi Crowther University, Oyo Town, Nigeria. Table 1 shows the total number of bacteria count which ranged from  $1.62 \times 10^6$  (Soil Sample E) to  $8.70 \times 10^6$  cfu/g (Soil sample H).

An amylase-producing bacterium is shown in Plate 1A. There was a clear zone around the line of streak of the bacterium which was due to its ability to hydrolyse starch. Plate 1B shows a bacterium with the ability to produce cellulase. The bacterium hydrolysed Carboxy Methyl Cellulose (CMC) agar around the line of streak as indicated by clear zone. A protease-producing bacterium is shown in Plate 1C. There was a clear halo along the line of streak which is as a result of the ability of the bacterium to hydrolyse skimmed milk agar.

**Table 1. Bacterial count of different soil samples**

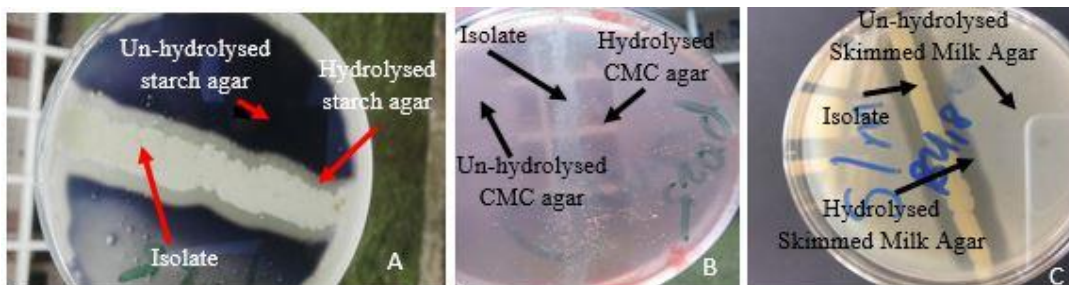
Soil sample	Total bacterial count (cfu/g)
A	$2.40 \times 10^6$
B	$1.72 \times 10^6$
C	$2.58 \times 10^6$
D	$5.80 \times 10^6$
E	$1.62 \times 10^6$
F	$2.52 \times 10^6$
G	$2.59 \times 10^6$
H	$8.70 \times 10^6$

The isolated bacteria belong to the Genera *Bacillus* (30), *Enterobacter* (6) *Staphylococcus* (3) and *Klebsiella* (3) as shown in Table 2. The highest relative amylase activities (2.88) was recorded in isolate SPD13 (*Enterobacter* sp.) which was followed by 2.86 in isolate SPD24

(*Bacillus* sp.). All the eight isolates that were able to produce amylase on plate were *Bacillus* spp. with the exception of isolates SPD13, SPD25 and SPD211 which were *Enterobacter* spp. Amylase-producing bacteria were isolated from four (B, D, F and H) out of the eight soil samples. Eleven of 42 isolated bacteria were able to produce cellulase on plate with relative activities ranging from 1.39 in isolate SBU12 (*Bacillus* sp.) to 4.50 in isolate SID21 (*Bacillus* sp.). Out of the eleven (11) cellulase-producing bacteria, eight (8) were *Bacillus* spp. while three (3) were *Enterobacter* spp. Cellulolytic bacteria were not isolated from soil sample A, C and G. Almost half (19 out of 42) of the isolated bacteria were able to produce protease on plate with relative activities of 1.21 (isolates SFK11 and SG11) to 5.17 (isolate SPD211). The nineteen proteolytic bacteria are made up of one *Staphylococcus* sp. (isolate SBU13), one *Klebsiella* sp. (isolate SFK11), three *Enterobacter* spp. (isolates SPD13, SPD25 and SPD211) and fourteen *Bacillus* spp. Eight (isolates SBU14, SBU18, SFK14, SID21, SPD13, SPD24, SPD25 and SPD211) out of 42 isolates were able to produce three extracellular enzymes (amylase, cellulase and protease) on plate. The eight amylolytic, cellulolytic and proteolytic bacteria are five (5) *Bacillus* spp. and three (3) *Enterobacter* spp. None of the bacteria isolated from soil samples A and C was able to produce any of the three enzymes (amylase, cellulase and protease) on plate.

### 4. DISCUSSION

The most frequent of all the bacteria isolated from soil samples in this study was *Bacillus* species which is in line with the work of Wang et al. [2] who reported that 80% of bacteria isolated from soil samples was *Bacillus* species. The



**Plate 1A. Amylase-producing bacterium on starch agar plate**  
**Plate 1B. Cellulase-producing bacterium on Carboxy Methyl Cellulose (CMC) agar plate**  
**Plate 1C. Protease-producing bacterium on skimmed milk agar plate**

**Table 2. Relative extracellular enzymes activities of bacteria isolated from soil samples**

Soil samples	Isolates	Relative enzymes activities			Probable organisms
		Amylase	Cellulase	Protease	
A	SAP211	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SAP212	0.00	0.00	0.00	<i>Bacillus</i> sp.
B	SBU12	0.00	1.39	1.68	<i>Bacillus</i> sp.
	SBU13	0.00	0.00	2.40	<i>Staphylococcus</i> sp.
	SBU14	1.25	1.40	1.50	<i>Bacillus</i> sp.
	SBU16	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SBU18	2.50	1.88	3.78	<i>Bacillus</i> sp.
C	SCA13	0.00	0.00	0.00	<i>Staphylococcus</i> sp.
	SCA14	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SCA15	0.00	0.00	0.00	<i>Staphylococcus</i> sp.
	SCA16	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SCA17	0.00	0.00	0.00	<i>Klebsiella</i> sp.
	SCA22	0.00	0.00	0.00	<i>Enterobacter</i> sp.
D	SFK11	0.00	0.00	1.21	<i>Klebsiella</i> sp.
	SFK12	0.00	0.00	1.38	<i>Bacillus</i> sp.
	SFK13	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SFK14	1.75	1.67	3.38	<i>Bacillus</i> sp.
	SFK15	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SFK21	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SFK22	0.00	0.00	3.27	<i>Bacillus</i> sp.
	SFK23	0.00	0.00	0.00	<i>Bacillus</i> sp.
E	SG11	0.00	1.88	1.21	<i>Bacillus</i> sp.
	SG21	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SG23	0.00	0.00	0.00	<i>Bacillus</i> sp.
F	SID12	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SID13	0.00	0.00	3.75	<i>Bacillus</i> sp.
	SID21	1.88	4.50	3.38	<i>Bacillus</i> sp.
	SID23	0.00	0.00	3.44	<i>Bacillus</i> sp.
	SID24	0.00	0.00	1.60	<i>Bacillus</i> sp.
G	SMU11	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SMU12	0.00	0.00	1.46	<i>Bacillus</i> sp.
	SMU13	0.00	0.00	0.00	<i>Klebsiella</i> sp.
	SMU21	0.00	0.00	0.00	<i>Bacillus</i> sp.
H	SPD12	0.00	0.00	0.00	<i>Enterobacter</i> sp.
	SPD13	2.88	3.00	2.00	<i>Enterobacter</i> sp.
	SPD14	0.00	1.50	1.31	<i>Bacillus</i> sp.
	SPD22	0.00	0.00	0.00	<i>Enterobacter</i> sp.
	SPD23	0.00	0.00	0.00	<i>Bacillus</i> sp.
	SPD24	2.86	1.50	4.29	<i>Bacillus</i> sp.
	SPD25	2.20	2.00	4.17	<i>Enterobacter</i> sp.
	SPD211	1.23	2.00	5.17	<i>Enterobacter</i> sp.
SPD212	0.00	0.00	0.00	<i>Bacillus</i> sp.	

predominant feature of *Bacillus* species could be as a result of endospores of *Bacillus* spp. which gave them better ability to survive under harsh environment. *Bacillus* species was also isolated from soil samples by some researchers [2,14,15]. Ability of Amylase-producing bacteria were observed with their ability to hydrolyse starch agar which is in line the work of Olanbiwoninu and Fasiku [5]. Bacteria with cellulolytic properties degraded carboxymethylcellulose in

carboxymethylcellulose agar which corroborates with the findings of Olanbiwoninu and Fasiku [5]. Proteolytic bacteria obtained in this work degraded skimmed milk agar as reported by Cui et al. [16].

In this research, bacteria that produce extracellular enzymes (amylase, cellulase and protease) were isolated from soil samples. Begum et al. [17] reported that soil bacteria have

potential of being unique and exploited for commercial and industrial use. Production of protease by *Bacillus*, *Enterobacter*, *Klebsiella* and *Staphylococcus* observed in this study has been reported [18-20]. Tondo et al. [18] utilized *Klebsiella oxytoca* isolated from milk sample for production of protease, Vandecandelaere et al. [19] produced protease using *Staphylococcus epidermidis* and Luang-In et al. [20] used *Bacillus*, *Staphylococcus* and *Enterobacter* species to produce protease.

Two Genera of bacteria (*Bacillus* and *Enterobacter*) were observed to have produced amylase, cellulase and protease together in this work. de Veras et al. [21] reported production of amylase, cellulase and protease by a single bacterium (*Bacillus subtilis* SR60). Jadhav et al. [22] reported amylase-producing *Enterobacter*, Kanchana et al. [23] obtained protease-producing *Enterobacter* and Akintola et al. [24] had cellulolytic *Enterobacter* but none of the *Enterobacter* used by these researchers was able to produce amylase, protease and cellulase together as obtained in this research. All bacteria with amyolytic properties were also able to produce cellulase and protease. Similar result was observed by Olanbiwoninu and Fasiku [5] who reported that all their amyolytic bacteria were also able to produce cellulase.

## 5. CONCLUSION

Bacteria with ability to produce amylase, cellulase and protease were isolated from soil samples. All amylase-producing bacteria were also able to produce cellulase and protease. Ability of a single bacterium to produce three enzymes could make such a bacterium relevant and important in enzymes and ethanol-producing industries.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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