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Isolation and Screening of *Bacillus subtilis* from Soil for Amylase Production

***Madika, A.[1], Ameh, J.B.[1] and Machido, D.A.[1]**

[1]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University Zaria

[*]Corresponding author: abubakarmadika@gmail.com, Phone: +2347061587391

Abstract

This study was aimed at isolating *Bacillus subtilis* from soil and screening the isolates for amylase production. A total of fifteen (15) soil samples, five each from botanical garden, refuse dump sites and flower beds were collected and subjected to cultural isolation followed by biochemical and microgen identification. The isolates of *B. subtilis* were then screened for amylase production using starch agar plate method. Nine (9) isolates were confirmed to be *Bacillus subtilis* with percentage occurrence of 80% from refuse dump sites and botanical garden and 20% from flower bed. All the *B. subtilis* isolates demonstrated amylase production ability with isolate RD4 from refuse dump sites having the highest diameter of zone of starch hydrolysis (29mm). *Bacillus subtilis* can readily be isolated from various soil types with frequency of 80% in soils from garden and refuse dumps and 20% from flower bed. All the *B. subtilis* isolates demonstrated the potential for amylase production. This preliminary study could provide information on the isolation of *B. subtilis* from various soil types for the production of amylase.

Keywords: *Bacillus subtilis*, screening, amylase, production

INTRODUCTION

Amylases are enzymes that catalyze the hydrolysis of starch into simple sugar units of maltose and glucose. Amylases are widely distributed in nature and can be derived from various sources such as plants, animals and microorganisms namely bacteria, fungi and actinomycetes (Pandey *et al.*, 2000). The bacterial amylases are derived from *Bacillus subtilis, B. amyloliquefaciens, B. licheniformis* and *B. stearothermophilus* (Bessler *et al.*, 2003). Amylases are of great significance and have biotechnological applications ranging from food, fermentation, detergent, pharmaceutical, brewing, textile and paper industries (Saxena and Singh, 2011).

Members of the genus *Bacillus* are heterogeneous and they are very versatile in their adaptability to the environment. Several factors influence the nature of their metabolic processes and enzymes produced. A great deal of attention is being given to thermophilic and extremely thermophilic microorganisms and their enzymes (Oyeleke and Oduwole, 2009). Many species of *Bacillus* produce a large variety of extra cellular enzymes, such as amylases, which have significant industrial importance such as in food, paper, detergent, textile and pharmaceutical industries.

Bacillus subtilis are rod-shaped, Gram-positive bacteria that are naturally found in soil and vegetation. They grow in the mesophilic temperature range with the optimal temperature between 25-35^{o}C. They form stress-resistant endospores that enable them survive under harsh environmental conditions (Bandow *et al.,* 2002). This study was aimed at isolating *Bacillus subtilis* from soil samples of different locations and screening the isolates for their ability to produce amylase.

MATERIALS AND METHODS

Collection of Samples

Fifteen (15) soil samples were collected from different locations namely, flower bed around the Department of Microbiology (FB), refuse dump site within the campus (RD) and the botanical garden of Department of Biological Sciences, Ahmadu Bello University, Zaria (BG). Five (5) samples (10 grams each) were separately collected from each location at a distance of 300 meters interval and a depth of 5-10cm. Each sample was packaged in a sterile bottle using a hand trowel and labelled appropriately (Ubalua, 2014). The soil samples were brought to the Industrial/Food Research Laboratory, Department of Microbiology, Ahmadu Bello University, Zaria for further analyses.

Isolation and Characterization of *Bacillus subtilis*

Isolation of *Bacillus subtilis* from the soil

Ten grams (10g) of each soil sample was suspended in 90ml of sterile distilled water. The soil suspension was heat shocked at 60^{o}C for one hour in a water-bath to kill non-spore forming organisms (Ubalua, 2014). A loopful each of the soil suspension was inoculated by streaking on nutrient agar medium. The inoculated plates were incubated aerobically at 37^{o}C for 24hrs and examined for the appearance of colonies. The colonies that exhibited cultural characteristics typical of *Bacillus* species i.e. round or irregular; thick and opaque; cream-colored colonies were sub-cultured onto nutrient agar slants for subsequent identification.

Identification of *Bacillus subtilis*

Bacillus subtilis isolates were primarily identified on the basis of taxonomic properties and microgen identification system. The characteristic morphological, cultural and biochemical properties were observed (Bergey, 2004; Cowan and Steel, 2003).

Cultural characterization

Isolates on nutrient agar plates were examined for size, pigmentation, form, margin and elevation of the colonies.

Morphological characterization

Morphological characteristics such as the cell shape, cell arrangement as well as the Gram's reaction of the organism were determined by Gram staining technique. Endospore-staining technique was also carried out to morphologically characterize the isolates.

Biochemical characterization

Biochemical tests such as catalase, motility, citrate, urease, indole, Methyl red, Voges-Proskauer, nitrate reduction, starch hydrolysis as well as sugar fermentation were carried out according to standard procedures.

Microgen Identification

Bacillus subtilis isolates were further subjected to microgen identification system following standard procedures (Microgen Bioproducts Ltd, U.K.).

Screening of the *Bacillus subtilis* Isolates for Amylase Production

The amylase production potential of the test isolates was determined by using the starch agar plate method. The identified *B. subtilis* isolates were inoculated centrally on starch agar medium. The inoculated plates were then incubated aerobically at 37^{o}C for 24hrs. After the incubation period, the cultured plates were flooded with Lugol's iodine solution and observe for zone of hydrolysis around the colonies. The diameter formed after the addition of iodine solution was measured in millimetres using a transparent ruler to represent the amylolytic activity (Oyeleke *et al*., 2011).

RESULTS

Isolation and Identification of *Bacillus subtilis*

Eleven isolates were confirmed to be *B. subtilis* based on cultural, microscopic as well as biochemical characteristics (Table 1). However, only nine isolates were further confirmed to be *B. subtilis* based on microgen identification system (Table 2). The percentage occurrence of the *Bacillus subtilis* isolates in the three soil samples is presented in Table 3 with soil samples from flower bed having the least isolation rate of 20%, while soils from botanical garden and refuse dump site had isolation rate of 80% each.

Amylase Production by *Bacillus subtilis* Isolates

The nine isolates were screened and found to be capable of producing amylase which was identified by zone of clearance signifying hydrolysis of starch on agar plate. The isolate named RD4 (from refuse dump) gave the highest (29mm) zone of starch hydrolysis while isolates named BG1 and BG3 (from botanical garden) gave the least (15mm each) zone of starch hydrolysis as presented in Table 4

Table 1: Cultural, Microscopic and Biochemical Characteristics of the *Bacillus* Isolates

Isolate's Code	Growth on NA	GRM	SS	Biochemical Characteristics									Sugar Fermentation				Tentative
				SH	M	C	Cat	U	I	MR	VP	NR	Glu	Man	Ara	Xyl	Identity
BG1	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*
BG2	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	-	*B. subtilis*
BG3	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*
BG4	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	-	+	*B. subtilis*
BG5	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*
RD1	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*
RD2	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*
RD3	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*
RD4	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*
FB1	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*
FB4	Creamy, opaque colonies	G+ve rods	oval	+	+	+	+	-	-	-	+	+	+	+	+	+	*B. subtilis*

Key: BG; Botanical garden, **RD**; Refuse dump, **FB**; Flower bed, **NA**; Nutrient agar, **GRM**; Gram reaction and Morphology, **SS**; Spore Staining, **SH**; Starch Hydrolysis, **M**; Motility, **C**; Citrate Utilization, **U**; Urease, **I**; Indole, **MR**; Methyl red, **VP**; Voges-Proskauer, **NR**; Nitrate Reduction, **Glu**; Glucose, **Man**; Mannitol, **Ara**; Arabinose, **Xyl**; Xylose, **G+ve;** Gram positive

Table 2: Identity of *Bacillus subtilis* Isolates based on Microgen

Isolate's Code	Octal Code	Probability (%)	Identity
BG1	70710427	99.55	*B. subtilis*
BG2	70710427	99.55	*B. subtilis*
BG3	70710427	99.55	*B. subtilis*
BG4	70710427	99.55	*B. subtilis*
RD1	73710427	99.68	*B. subtilis*
RD2	70710427	99.55	*B. subtilis*
RD3	70710427	99.55	*B. subtilis*
RD4	70710427	99.55	*B. subtilis*
FB4	73710427	99.68	*B. subtilis*

Key: BG; Botanical garden, **RD**; Refuse dump, **FB**; Flower bed

Table 3: Occurrence of *Bacillus subtilis* in Various Soil Samples Collected

Sampling Location	Number of Samples Collected n = 15	Number Positive (%)
Botanical garden	5	4(80)
Refuse dump site	5	4(80)
Flower bed	5	1(20)
Total	15	9(60)

Key: n = Total number of samples collected

Table 4: Amylase Production by *Bacillus subtilis* Isolates

Isolate Code	Diameter of Zone of Starch Hydrolysis (mm)
BG1	15
BG2	17
BG3	15
BG4	20
RD1	22
RD2	20
RD3	22
RD4	29
FB4	22

BG; Botanical garden, **RD**; Refuse dump, **FB**; Flower bed

DISCUSSION

Eleven (11) isolates were presumed to be *B. subtilis* based on cultural, morphological as well as biochemical characteristics, out of which only nine (9) were confirmed to be *B. subtilis* based on microgen identification. The reduction in the number of confirmed isolates could be due to the sensitivity of the microgen system of identification. Similarly the differences observed in the octal code of the isolates and hence the percentage probability could be due to strain variations. Out of the fifteen soil samples collected, a percentage occurrence of 80% was obtained with soils from botanical garden and refuse dump site whereas soil from flower bed had the least percentage occurrence of 20%. This might be due to the richness in organic matter content of the humic soil from the refuse dump and botanical garden, whereas, the flower bed might have little organic matter as nutrient to the organism. This agrees with the findings of Bello (2016) who reported a higher isolation rate (50%) from organically-rich soil than from nutrient poor soil (20%). This is also similar with the findings of Ubalua (2014) who reported a higher occurrence of *B. subtilis* from garden soil.

Bacillus species are considered to be the most important sources of amylase and have been used frequently for enzyme production. Out of nine (9) isolates confirmed to be *B. subtilis* and screened for amylase production ability, the isolate from refuse dump (RD4) had the highest zone of starch hydrolysis (29mm) whereas, the lowest zone of starch hydrolysis (15mm) was observed with isolates named BG1 and BG3 from botanical garden. This observed variation might be due to differences in the metabolic capabilities of the different isolates. This result is in agreement with the work of Ubalua (2014) where out of the 13 isolates observed, isolate CNS_3 (*Bacillus subtilis*) demonstrated highest starch hydrolyzing ability. Similar result was also reported by Vijayalakshmi *et al*. (2012) where isolate KC3 had the highest diameter zone of starch hydrolysis (23mm) on starch agar plate. The occurrence of amylolytic organisms in soil agrees with the earlier report of Omemu *et al*. (2005), which described soil as a repository of amylase.

CONCLUSION

Bacillus subtilis can readily be isolated from various soil types with frequency of 80% in soil samples from botanical garden and refuse dump and 20% in soil samples from flower bed. All the isolates of *B. subtilis* obtained from the three soil sources demonstrated the ability for amylase production.

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