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Bacillus Amylase

Preliminary Investigation of Amylase Producing-Bacteria from Soil in Gombe Metropolis

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ABSTRACT

Amylases are enzymes that are able to hydrolyse starch or glycogen molecules into polymers of glucose units. They have great potential applications in various industrial processes like in pharmaceutical, fermentation and food industries. Research on starch degrading enzymes has resulted into increased applications of amylases in different industrial processes. These enzymes occupy a greater space in the current biotechnological processes such as detergent, starch degradation, pharmaceutical, foodstuff, textile, and paper manufacturing. In fact, amylases constitute nearly 25% of the total sale of global enzymes. Amylases have been screened and identified from various sources, both eukaryotic and prokaryotic organisms such as animals, plants, fungi and bacteria, respectively. To further isolate novel amylases with enhanced desirable properties for such diverse industrial application, more organisms need to be screened. In this study, a total of 27 bacterial isolates were isolated from soil samples in Gombe metropolis. The bacteria were screened for amylase production using plate screening method. Each isolate was streaked onto a 1% starch agar plate and incubated for 24h at 37 °C. The plates were covered with iodine solution and observed for positive amylase isolates based on the formation of clearing zones against the blue black background. The results confirmed eight (8) isolates of amylase-producing bacteria which include *Bacillus subtilis, Escherichia coli*, *Streptococcus* spp., *Salmonella* spp., *Pseudomonas* spp., *Serratia* spp., *Proteus vulgaris*, and *Klebsiella* spp. In conclusion, bacterial isolates capable of amylase production have been successfully screened and identified. This research may serve as a stepping stone to isolating functional amylase enzymes from these bacteria for promising industrial applications.

INTRODUCTION

Amylase enzymes are able to hydrolyse starch or glycogen molecules into polymers of glucose units. Starch in particular, constitutes the major constituents of daily diet as food residues and food stains found on dishes and clothes, respectively. The discovery of these enzymes has led to their increased applications in several industrial processes. The most important sub-groups involved in many biotechnology applications such as detergent, starch degradation, pharmaceutical, foodstuff, textile, and paper manufacturing are α-amylase, β-amylase and glucoamylase (GA) [1]. Additionally, these enzymes have a wide range of applications in several other fields like clinical, medical and analytical chemistry. Amylases have been reported from different sources such as plants, animals and microorganisms. Many amylases of microbial origin have been commercially available to meet the increasing industrial demands [1]. They have almost substituted the already existing chemical means of starch hydrolysis in the industrial starch processing [2]. The key advantage that makes microorganisms to be attractive for amylase production is their economical large production capacity as well as the amiability of microbes to manipulations such as mutations, strain improvement, and other changes through genetic engineering or similar means employed to optimize amylase production or to produce enzymes of most desired characteristics [3,4,5].

Being it the main ingredient of the human diet, starch is processed by chemical and enzymatic means into several products such as glucose syrups, starch hydrolysates, fructose,

maltodextrin derivatives or cyclodextrins for food industrial applications. Furthermore, ethanol can be produced from the sugars formed through fermentation process. Even though many plants could be source of starch, only a few of them are utilized in the industrial starch processing. The most important industrial sources include maize, potato, tapioca and wheat. However, several limitations such as thermal resistance, low shear resistance, thermal decomposition as well as high tendency to retrogradation may hinder its usage in certain industrial food applications [4,6,7]. Although amylases have been reported by several researchers, there was no report on these enzymes from our study area. In this research amylase producing bacteria have been successfully screened and identified from soil samples in Gombe metropolis.

MATERIALS AND METHODS

Collection and preparation of soil samples

Samples collection and preparation was carried out according Singh *et al*. [1] with a slight modification. Soil samples were collected from Gombe metropolis in sterile polythene bags and transported to Microbiology laboratory of the department of Microbiology, Gombe State University for processing. One gram (1 g) of the soil was diluted in 9 mL of sterile distilled water and thoroughly shaken. One milliliter (1 mL) was taken from the stock solution and serially diluted (Ten-fold dilution) in six (6) test tubes each containing 9 mL of distilled water [8].

Isolation and Screening of amylase-producing bacteria

An aliquot of 0.1 mL of each dilution was inoculated on nutrient agar plate using pour plate technique and incubated for 24 h at 37 °C. Pure culture of individual colonies was streaked on nutrient agar plate supplanted with 1% soluble starch and incubated for 24 h at 37 °C. After incubation, the plates were covered with an iodine solution **(**0.5 mL**)** and observed for clear zones formation around the colonies [1].

Identification of Positive amylase-producing bacteria

Positive amylase-producing bacteria were identified based on Gram staining reaction and different biochemical tests (Indole test, citrate utilization test, Catalase test, Oxidase test, Triple Sugar iron test, Urease test) according to Cheesbrough [8].

RESULTS AND DISCUSSION

Isolation and screening of amylase-producing bacteria

A total of 27 bacteria have been isolated from the soil and screened the potential amylase producers on starch agar plates as described in the methodology. The positive isolates produced clear (halo) zones around their colonies upon the addition of iodine which indicates their ability to hydrolyse starch while the amylase negative isolates did not show any halo zones as shown in **Fig. 1**.

Identification of positive amylase producing-bacteria

The amylase positive isolates were subjected to Gram staining for their morphological characteristics and identified as *Bacillus subtilis, Escherichia coli*, *Streptococcus* spp*., Salmonella* spp., *Pseudomonas* spp., *Serratia* spp*., Proteus vulgaris*, and *Klebsiella* spp (**Table 1**). *B. subtilis* and *Pseudomonas* spp. had the highest occurrence each with a total of two (2) isolates while the remaining bacteria each has an occurrence of one (1) isolate (**Table 2**).

Fig 1. Positive (a) and negative (b) amylase producing bacteria on nutrient agar plates supplemented with 1% starch after 24h incubation at 37 °C. G3 indicate source of the soil sample.

Table 1. Morphological and biochemical characteristics of the bacteria.

Morphology and Biochemical tests												Bacteria
G1	M	C1	I	O	C2	U	G2	L	S	G3	H	
+	+	+	-	-	+	-	+	-	+	+	+	*Bacillus subtilis*
-	+	+	+	-	-	-	+	+	+	+	-	*Escherichia coli*
-	-	+	-	-	+	+	+	+	+	+	-	*Klebsiella* spp.
-	+	+	+	-	+	+	+	-	+	+	+	*Proteus* spp.
-	+	+	-	+	-	-	-	-	-	+	-	*Pseudomonas* spp.
-	+	+	-	-	-	-	+	-	-	-	+	*Salmonella* spp.
-	+	+	-	-	+	+	+	+	+	-	-	*Serratia* spp.
+	-	-	-	-	-	-	+	+	+	-	-	*Streptococcus* spp.

Key: G1= Gram reaction, M=Motility, C1=Catalase, I=Indole, O=Oxidase, C2=Citrate, U=Urase, G2=Glucose, L=Lactose, S=Sucrose, G3=Gas, H=Hydrogen sulphide

Table 2. Occurrence of Amylase-producing bacteria.

S/N	Bacteria	Occurrence (%)
1	*Bacillus subtilis*	2(20)
2	*Pseudomonas* spp.	2(20)
3	*Escherichia coli*	1(10)
4	*Klebsiella* spp.	1(10)
5	*Proteus* spp.	1(10)
6	*Salmonella* spp.	1(10)
7	*Serratia* spp.	1(10)
8	*Streptococcus* spp.	1(10)
	Total	10(100)

As a starch-degrading enzyme, amylase has great economic benefits, biotechnological and industrial significance. While rapid growth with metabolite production remains crucial parameters for selecting microorganisms as enzymes sources, other leading factors may necessitate how microorganisms could serve as principal enzyme source. Microorganisms succumb to physiological and physicochemical control, high product yield in comparison to plant or animal sources, tranquil recovery in downstream process, cost effectiveness during processing among other reasons. Furthermore, the raw materials, substrates, as well as production systems are collectively cheap. A number of developed and developing countries of the world are famous commercial producers of enzymes from microorganisms [9].

The application of nutrient agar plates amended with starch and iodine to detect amylase producing microorganisms based on clearing zones surrounding their colonies has been well documented by several researchers such as Forgarty and Kelly [10], Iverson and Millis [11], and Singh *et al*. [1]. Like in these reports, the screening results in our study showed the ability of the isolated bacteria to produce halo zones on nutrient agar plates supplemented with soluble starch.

CONCLUSION

In conclusion, the present study screened and identified ten (10) positive amylase producing bacteria from soil in Gombe metropolis for the first time to the best of our knowledge. The bacteria may be used to study functional amylase enzymes for their potential industrial applications.

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