NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 49 Number 2, October 2018. Pp. 85-88
Available online at: http://www.ajol.info/index.php/naj

PRELIMINARY INVESTIGATION OF AMYLASE-PRODUCING BACTERIA FROM SOME CASSAVA FARMS IN UMUDIKE, ABIA STATE, SOUTH-EAST NIGERIA

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ABSTRACT
Amylase producing microorganisms are richly distributed in environment where cassava is processed or where it is being cultivated. Isolation and identification of these microorganisms may be a step forward in providing ways of converting cassava into value added products for the benefit of man and farm animals. In this research, soil, water and air samples of three cassava farms located in Umudike community were examined for the presence of amylase producing bacteria. Isolation of these bacteria was carried out by culture plate method. The pure isolates were identified on the bases of their colonial morphology, microscopy and biochemical tests. The isolates obtained were mainly environmental contaminants though the gram positive isolates may possibly be used as starter cultures in cassava processing due to the possession of amylase but the gram negative isolate may not possibly be used due to the absence of amylase. *Psuedomonas sp.* was the only gram negative isolate identified while *Bacillus sp., Corynebacterium sp. and Micrococcus sp.* were the gram positive isolates identified. *Bacillus sp.* was the most prevalent isolate in all the samples investigated compared to the other isolates identified. We suggest that effort be made to validate these preliminary findings of the potentiality of these gram positive isolates identified as amylase producing bacteria in future studies.

Keywords: Amylase producing bacteria, and Cassava farms

Introduction

Processing of starch into value added products like floor, glucose syrup, additives in detergents, among other products is partly attributed to amylase producing microorganisms which have the ability to degrade starch in different ways (Vijayan *et al.*, 2015). Amylases are enzymes that break down starch or glycogen and are produced by a variety of living organisms, ranging from bacteria to plants and humans (Kathiresan and Manivannan, 2006). Amylases are classified based on how they break down starch molecules. Alpha-amylase reduces the viscosity of starch by breaking down the bonds at random, therefore producing varied sized chains of glucose. (Ashwini *etal.*, 2011). Beta-amylase on the other hand, breaks the glucose-glucose bonds down by removing two glucose units at a time, thereby producing maltose (Yuguo *et al.*, 2000). Amyloglucosidase (AMG) breaks successive bonds from the non-reducing end of the straight chain, producing glucose (Pandey *etal.*, 2000). The microbial amylases are preferred to other sources because of their cost effectiveness; plasticity and vast availability hence, have been utilized in some industrial processes such as saccharification of starch for the production of alcohol production, high fructose corn syrup, additives in detergents to remove stains, among others (Patel, 2015). In this research, a preliminary investigation of amylase-producing bacteria from some cassava farms in Umudike Abia State was carried out which may serve as a step forward in the search for suitable indigenous amylase- producing bacteria for cassava processing.

Materials and Methods

Collection of samples

Soil, water and samples were collected from three farms located in Umudike community of Abia State, South East Nigeria.

Isolation of amylase producing bacteria

Isolation of the amylase producing bacteria was carried out in triplicates according to standard method adopted by Vaidya and Rathore (2015) with slight modification. Serial dilution of each soil sample was made and plated on freshly prepared starch nutrient agar plate containing 2% of starch. 0.1ml of the diluted soil sample each was inoculated on the media then incubated at 37°C for 24 hours while 0.1ml of

the water sample each, was inoculated on freshly prepared starch nutrient agar plate and was incubated at 37°C for 24 hours. Another plate of freshly prepared starch nutrient agar was exposed to air at the three farms at specific locations for 20-30 minutes and incubated at 37°C for 24hours.

Identification of amylase producing isolates

The pure isolates were identified based on their colony morphology i.e. colour, shape, size and nature of colony according to Bergey's manual of systematic bacteriology (2012) then subjected to series of biochemical tests namely Gram-staining, Motility test, Oxidase test, Gelatin liquefaction, IMVIC test and Sporulation test.

Screening for Amylase Activity (Starch Iodine Test)

This was carried out in triplicates according to standard method adopted by Patel (2015) with slight modification. The isolated colonies were picked up from each plate containing pure culture after subsequent sub culturing onto freshly prepared starch agar plates and then streaked on another freshly prepared starch agar plates. After incubation at 37°C for 24-48 hours, individual plates were flooded with Gram's iodine to produce a deep blue coloured starch-iodine complex. In the zone of degradation no blue colour forms, which is the basis of the detection and screening of an amylolytic strain.

Results and Discussion

Twelve pure isolates were obtained after sub culturing onto the freshly prepared starch-nutrient agar plates and their cultural morphology and gram-stained microscopic description were summarized in the Table 1. Out of the twelve pure isolates obtained from the various samples after flooding with grams iodine, eleven, revealed clear zones of starch hydrolysis indicating the presence of amylase while one of them revealed no clear zone of starch hydrolysis indicating the absence of amylase. Amylase-positive isolates that were subjected to the biochemical tests were identified as *Bacillus sp.*, *Corynebacterium sp.* and *Micrococcus sp.* while the amylase-negative isolate was identified as *Pseudomonas sp.* as shown in Tables 1, 2 and 3. Isolates obtained were similar to those isolated by (Pandey, 2000 and Zambare, 2010) as starch hydrolyser's. The low incidence of Gram negative bacteria isolates from the samples can be related to the inability of most strains to degrade starch due to the absence of amylases in contrast to Gram positive bacteria which possess amylases in most strains. The prevalence of *Bacillus sp.* in all the investigated samples compared to the other isolates may be due to its ability to form easily dispersed spores in air, water and soil, usually during adverse conditions, as similarly documented by (Vaidya and Rathore, 2015). Although many microorganisms produce amylase which degrades starch in cassava, the ones most commonly used for industrial purposes are *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquifaciens* and *Aspergillus niger*, which may be attributed to the high quality of their amylases compared to other microorganisms that degrade starch (Tonkova, 2006). The results of this preliminary investigation suggests that the gram positive bacteria present in these cassava farms may possible be a source of indigenous amylase which can be isolated, purified and scaled up for industrial purposes, however, efforts should be made to validate this preliminary findings.

Conclusion

The isolates obtained were mainly environmental contaminants though; the gram positive isolates may probably be an indigenous source of amylase for the production of some value added products. *Bacillus sp.* was the most prevalent in these samples probably due to its ability to sporulate in adverse conditions compared to the other isolates. Effort should be made to validate these preliminary findings of the potentiality of these gram positive isolates as amylase producing bacteria for subsequent studies.

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Figure 1: *Bacillus sp.* (UP) and *Corynebacterium sp.* (DOWN), from one of the Soil sample after flooding with gram's iodine

Figure 2: *Bacillus sp.* (UP) and *Micrococcus sp.* (DOWN), from one of the Water sample after flooding with gram's iodine

Figure 3: *Bacillus sp.* (UP) and *Pseudomonas sp.* (DOWN), from one of the Air samples after flooding with gram's iodine

Table 1: Identification of air Isolates

Code	Gram-stain	Starch hydrolysis	Motility test	Catalase test	Oxidase test	Gelatin liquefaction	IMVIC test	Sporulation test	Isolate
S1	+	+	+	+	-	-	+	+	*Bacillus sp.*
S2	-	+	+	-	+	+	-	-	*Psuedomonas sp.*
S3	+	+	-	+	-	-	-	-	*Corynebacterium sp.*
S4	+	+	+	+	-	-	+	+	*Bacillus sp.*

Table 2: Identification of soil isolates

Code	Gram-stain	Starch hydrolysis	Motility test	Catalase test	Oxidase test	Gelatin liquefaction	IMVIC test	Sporulation test	Isolate
A1	+	+	+	+	-	-	+	+	*Bacillus sp.*
A2	+	+	-	+	-	-	-	-	*Micrococcus sp.*
A3	+	+	-	+	-	-	-	-	*Corynebacterium sp.*
A4	+	+	+	+	-	-	+	+	*Bacillus sp.*

Table 3: Identification of water isolates

Code	Gram-stain	Starch hydrolysis	Motility test	Catalase test	Oxidase test	Gelatin liquefaction	IMVIC test	Sporulation test	Isolate
W1	+	+	-	+	-	-	-	-	*Micrococcus sp.*
W2	+	+	-	+	-	-	-	-	*Corynebacterium sp.*
W3	+	+	+	+	-	-	+	+	*Bacillus sp.*
W4	-	+	+	-	-	+	-	-	*Psuedomonas sp.*

Note: A= Air W= Water S= Soil, += Positive - = Negative IMVIC= Idole, Methyl red, Voges-Proskauer, Citrate.