Studies on the Potential of *Rhizopus* species from Raw Food and Soil for Amylase Enzyme Production

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Abstract: *Rhizopus* species from potato, millet and soil samples were isolated and screened for their ability to produce enzyme amylase. Potato and millet were dried, grinded and the samples including soil were serially diluted and spread plated (0.1ml) on sterile potato dextrose agar (PDA) platesincubated at 35°C for 5days. Colonies were repetitively sub-cultured in order for pure cultures. Fungi *Rhizopus* was macroscopically and microscopically identified based on standard procedures. *Rhizopus* species were screened using agar plate method at which hydrolysis zones were observed and measured in millimeter (mm) by meter rule. Enzyme was quantified by solid state fermentation (SSF) during which wheat bran (the substrate/medium) and time (96hrs) was used for production. For enzyme extraction, the mixture of fermented medium and tween 80 (0.1%) was shaken by rotary shaker, squeezed by muslin cloth and filtered through filter paper (Whatman No. 1). For enzyme activity determination; crude extract of enzyme was mixed with starch, Sodium Chloride (NaCl) and phosphate buffer and the mixture was incubated in a water bath for 30mins at 40°C, DNSA was added to stop the reaction, heating the mixture again for 5mins after which distilled water was added and the absorbance 540nm was taken using spectrophotometer. About three (3) *Rhizopus* species were isolated from millet including *R. microsporus*, *R. nigricans*, and *Rhizopus* specie with zone of hydrolysis 31.0, 28.6 and 28.2mm and their enzyme activity 76, 52 and 48% respectively. Subsequently, only four (4) *Rhizopus* species were isolated from soil.They include*R. oryzae*, *R. americanus*, *R. oligosporus* and *R.nigricans* with hydrolysis zone 27.4, 25.3, 25.0, 22.0mm in diameter and enzyme activity 50, 45, 46 and 36% respectively.Similarly, about three (3) *Rhizopus* species were isolated from potato.They include*R. oligosporus*, and two (2) *Rhizopus* species with zone of hydrolysis 22.8, 20.7, 19.5mm and enzyme activity 40, 38 and 29% respectively. This reveals fungi *Rhizopus* can be isolated from varied raw food sources and soil with strain *R. microspores* isolated from millet having greater potential of producing this amylase enzyme.
Key words: *Rhizopus*, Screening, amylase and Production

INTRODUCTION

Amylase term was designated to enzymes able to degrade bulk sugars and their products like amylopectin, glycogen and others by hydrolysis of α- 1,4-glycosidic bonds (Prasanna, 2005; Alonazi *et al*., 2021). Amylase are enzymes which catalyses the breakdown of complex sugars e.g. starch into simple sugars like glucose, fructose and others useful in the industries such as detergent, paper, textiles, medicinal drug and many food producers (Ferreira *et al*., 2015).Studies have shown the availability of amylase in plants, animals and microbes (Sagu *et al*., 2019; Alonazi *et al*, 2021) while manufacturers are highly interested in microbial amylases because of their easier isolation in large quantity, low production cost and time, strong effectivity, steadiness to severe situations, less harmful and unchanging character (Costa de freitas*et al*., 2014, Gopinath *et al*., 2017).

A significant microbe *Rhizopus* is a genus of common saprobic fungi found on plants and some animals. They are found on a wide variety of organic substrates, including soil, grains and tubers (Ahmad *et al*., 2019). *Rhizopus* are well-recognized to consume varied carbon resources and many are not dangerous, on the other hand called GRAS (generally regarded as safe) organisms (Rhani and Ghosh, 2011; Londoño-Hernández *et al*., 2017). They are potential sources and good suppliers of bioproducts including enzymes (AbdRazak *et al*., 2015; Londoño-Hernández *et al*., 2017).

Among the other relevance of amylase include liquefaction as thinning agent used in starch technology, manufacture of maltose, maltooligomer mix, maltotetraose syrup, textile desizing, wastewater (starch)

treatments and direct conversion of starch to ethanol (Prasanna, 2005). The study was aimed at identifying the most productive *Rhizopus* sp from various sources for enzyme amylase production.

MATERIALS AND METHODS

Collection of Samples

Samples of wheat bran, potato and millet were obtained from Yelwa Market, Bauchi town and soil sample was collected from Abubakar Tafawa Balewa University (ATBU) Bauchi, football field. Each sample was obtain in a clean polyethylene bag and transported to the laboratory.

Isolation and Identification of *Rhizopus species*

Fungi were isolated from samples of soil (Bello *et al*., 2020), millet and potato used the method described by Makun*et al*. (2007). The samples were serially diluted and spread plated 0.1ml on sterile potato dextrose agar (PDA) plates (in duplicates) added with 0.5mg/ml streptomycin (Abdullahi *et al*., 2020) and incubated at 35°C for 5days. These were further subcultured repeatedly on fresh medium until pure colony was obtained. The colony of each was emulsified on separate clean slidecontaining a drop of lactophenol blue dye. These were viewed at ×40 0bjective lens using microscope. Fungi *Rhizopus*sp was identified microscopically based on the morphological standard procedure as described by Vijayaraghavan *et al*. (2011). *Rhizopus*sp. was maintained on potato dextrose agar (PDA) slant at 4°C for further use.

Screening of *Rhizopus* species for Amylase Production

Following the method described by Ikram-ul-Haq *et* al. (2002), *Rhizopus* species were screened for amylase activity on starch agar plate method; A pure colony was picked from the cultured Potato Dextrose Agar (PDA) and sub-cultured on PDA containing 2% of starch by stabbing, incubated at 35°C for 36 hours. Following flooded iodine solution, the culture plate was observed for zone of hydrolysis around the colony by showing clear zone around the colony. Meter rule was used to measure (mm) the clear zone in diameter.

Quantitative screening uses solid state fermentation (SSF) for α-amylase production as described by Omemu*et al.* (2015); conical flasks 500ml containing wheat bran 0.1kg and basal medium (peptone 0.005kg, K_2HPO_4 0.002kg, yeast extract 0.00025kg, $MgSO_4.7H_2O$ 0.0001kg and distilled water) 100ml. At pH 6.5 the mixture was alkaline. The wet medium was autoclaved at 121°C for 15mins. Exactly 10ml distilled water was transferred to sporulated PDA culture to form spore suspension. The inoculum 0.5ml contained about 10^5 spore suspensions by haemocytometer was pipetted into amylase production medium, incubated at 35°C for 4days(Omemu *et al*., 2015).

Enzyme Extraction

In taking out the enzyme, method by Omemu*et al.* (2004) was adopted. Fermented medium was mixed in tween-80 (0.1%) solution, the mixture was shaken on a rotary shaker at 28°C, 180rpm for 1hour. Muslin cloth was used to squeeze the resultant solution. These were filtered via filter paper (Whatman No. 1) and the resultant filtrate was an enzyme.

Determination of Amylolytic Activity

One (1) ml each of crude extract (enzyme) was pipetted into test-tube, and 1ml of 1% soluble starch containing sodium chloride and phosphate buffer (pH 6.5) was added. Then the mixture was incubated in a water bath at 40^0c for 30mins. The reaction was stopped by addition of 2ml dinitrosalicylic acid. The mixture was heated for 5 minutes in water bath after which 20ml distilled water was added and absorbance at 540nm was measured using spectrophotometer.

RESULT AND DISCUSSION

Three (3) *Rhizopus* sp. were isolated from millet; they include *R. microsporus*, *R. nigricans*, and *Rhizopus* species. Similarly, four (4) *Rhizopus* sp. were isolated from soil; *R. oryzae*, *R. americanus*, *R. oligosporus* and *R. nigricans.* Lastly, three(3) *Rhizopus* sp. frompotatoe; *R. oligosporus*, and two (2) *Rhizopus* species

were isolated. These were screened for ability to produce amylase enzyme. A strain from a particular sample had greater amylase production. Species of *Rhizopus* obtained from millet sample showed maximum hydrolysis zone 31, 28.6 and 28.2mm in diameter and their amylase activity were 76, 52 and 48% at four (4) days respectively (figure 1). *Rhizopus* species from millet showed higher ability of amylase production with wider inhibition zones *R. microsporus* 31.0 mm in diameter and larger enzyme activity 76% determined as compared to others. Millet is a food source (habitat) with higher polysaccharides (starch) contents (Annor *et al.*, 2017) and *Rhizopus* from that habitat may adapt complex sugar dissociation with mechanism for enzyme production faster than other species from different locale. The substrate (wheat bran) and conditions (pH, time and others) are the same while determining its activity. Food substances with greater starch quantity like maize were reported by Ayogu and Amadi (2009) that *Rhizopus* sp produced amylase.

Hydrolysis zone by *Rhizopus* from soil sample 27.4, 25.3, 25, 22mm in diameter and their relevant amylase activity were 50, 45, 46 and 36% at four (4) days respectively (figure 2). Soil has been a versatile habitat of microbes including fungi *Rhizopus* and was confirmed to produce amylase enzyme as reported by El-Abyad *et al.* (1999), Sun *et al.* (2010). Similar results were reported by Shi *et al.* (2010) identified the amylase enzyme produced by *Rhizopus* sp for its ability to break down corn starch substance. *Rhizopus* sp from potato sample had minimum zone of hydrolysis 22.8, 20.7, 19.5mm in diameter with amylase activity 40, 38 and 29% respectively (figure 3). Similar report of *Rhizopus* sp found in potatoes was by Agu *et al.* (2015). Fungi *Rhizopus* has been determined as significant agent for amylase production as revealed by different studies including Peixoto*et al.* (2003), Liao *et al.* (2007), Ferreira *et al.* (2015). The study showed the potential of *Rhizopus* sp in the enzyme production specifically amylase and that several starch/sugar containing compounds like grains, tubers and others are innate source of *Rhizopus* sp.

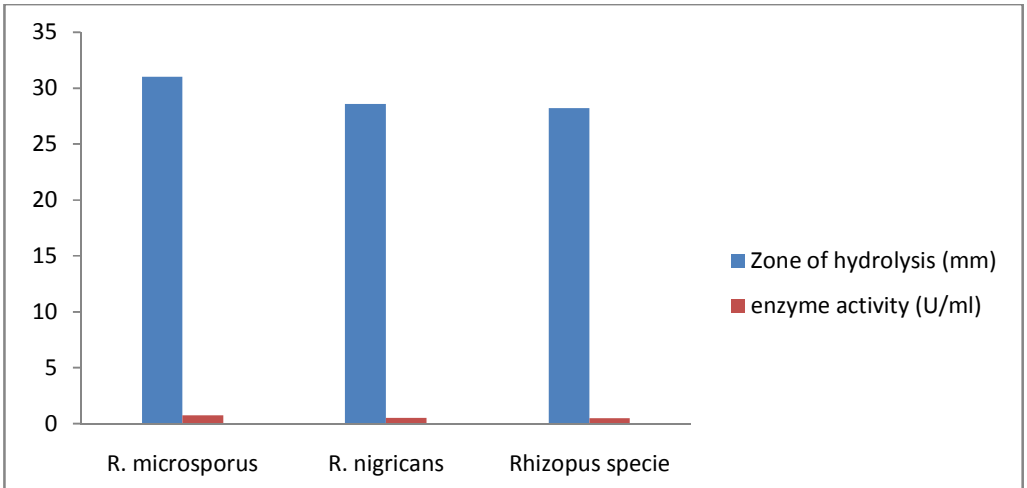

Figure 1: Amylase Activity of *Rhizophus* Isolated from Millet

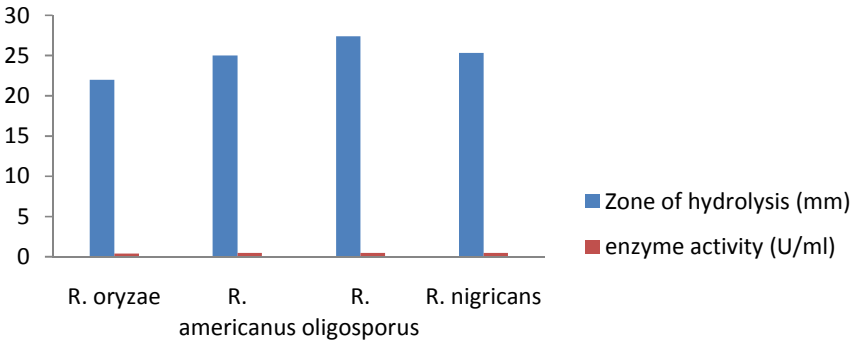

Figure 2: Amylase Activity of *Rhizophus* Isolated from Soil

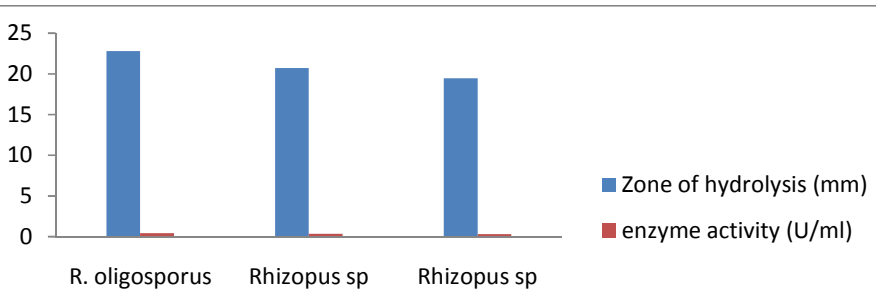

Figure 3: Amylase Activity of *Rhizophus* Isolated from Potatoe

CONCLUSION

Among the *Rhizopus* sp. screened from samples used; the strain isolated from millet showed higher ability to produce enzyme amylase with *R. microsporus* and *R. nigricans* observed. This indicated many starchy/sugar contained food substances accommodate *Rhizopus* sp. which showed higher potential to produce large quantity of enzyme amylase.

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