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Research Article

Amylase and Protease Activities of Microorganisms Isolated from Cassava Wastewater Contaminated Soil

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A B S T R A C T

Agro waste management is one of the problems faced by humans in their environment especially in many developing nations. In Nigeria, several agro wastes are generated in enormous quantities including cassava processing wastewater. Nigeria is the world-leading producer of cassava and it has been estimated that 5.142 million tonnes of cassava wastewater are discharged into the Nigerian environment of which 45–65% could be recovered. It has also been projected that the wastewater could increase significantly before 2035. The cassava wastewater is often discharged without any formal treatment methods by *gari* processors in many areas of cassava producing communities in Nigeria. Untreated cassava wastewater leads to environmental degradation and loss of biodiversity due to its lethal characteristics. Cassava wastewater can be harnessed through its use in biotechnological advances especially enzyme production. This study was designed to assess the amylase and protease activities of some microbial isolates (*Escherichia coli, Staphylococcus aureus, Pseudomonas, Bacillus, Micrococcus, Proteus,* and *Enterobacter* species viz: bacteria, and *Saccharomyces cerevisiae, Aspergillus, Mucor, Penicillium* and *Rhizopus* species viz: fungi) from cassava wastewater contaminated soil. The protease and amylase activities from the isolates were carried out using standard microbiological processes. The results showed that all the microbes have amylase and protease activities except for *Escherichia coli* and *Enterobacter* species, and *Proteus* and *Micrococcus* species showed only amylase and protease activity, respectively. These enzymes (amylase and protease) can be applied in numerous industrial sectors in the nation's economy. As such, microbes from cassava wastewater contaminated soil could be obtained and used for the production of useful enzymes with biotechnological potentials.

Keywords: Amylase, Cassava mill effluents, Enzymes, Microorganisms, Protease

Introduction

Enzymes are biomolecules produced by living organisms that help to accelerate various biological/ biochemical reactions that are pivotal in sustaining human life. Enzymes are efficient and specific biocatalysts that can speed up reaction rates several times faster than normal chemical reactions both in and out of the cell. Enzymes are present in all living things ranging from plants to animals including microorganisms. However, in plants and animals enzymes are produced in smaller quantities and are rarely exploited for industrial uses whereas microbial enzymes have found widespread uses in industries. This is because microbial enzymes are more active and stable, easier to handle, rapid multiplication and produce high yield, cheap to produce and are more susceptible to genetic manipulation.

Industrial uses of microbial enzymes are gaining more attention due to their eco-friendly and non-toxic nature. Other special characteristics such as thermotolerance, stability over different ranges of temperature and pH, and other harsh conditions have made microbial enzymes very useful in different commercial industries. Enzymes are generally biosynthesized from microbes that have advanced in biotechnology such as recombinant DNA technology, protein engineering, and metagenomics which have revolutionized the commercialization of enzymes with new activities and adaptabilities further increasing their use in different industrial processes.[1] Enzymes are useful in the manufacturing sector such as textile, food, pharmaceuticals, cleaning agents, biofuel, etc. These applications make microbial enzyme market very competitive. One of the most useful enzymes in industries is hydrolases which catalase the breakdown of molecules. Enzymes such as amylase and protease have been widely studied with the advent of biotechnology due to their extracellular hydrolytic properties.

Amylase is used as an additive in several production processes including pharmaceutical, food, fermentation, textile, paper,[2-4] detergents,[3] brewing and distilling industries, fine-chemical, clinical, medicinal and analytical chemistries.[2] Similarly, amylase has also found applications in breweries and biofuel industries.[5] Alpha-Amylase is also a useful conversion of starches into oligosaccharides.[3] Starch-converting enzymes are useful in the production of maltodextrin, glucose, and fructose syrups.[3] Proteases, on the other hand, have found applications in detergent production, food enzymes (used for baking, brewing, cheese production) meat tenderization, leather, textiles and fabrics[6,7] pulp, paper making industry, bioremediation processes[7] production of protein hydrolysates, solubilization of keratin materials to convert waste materials into useful products, in silver recovery from conventional gelatin-containing photographic film, liquefaction of organic waste and it can even be consumed by humans and animals and used as therapy during thrombosis and cancer treatment.[6]

Cassava wastewater contains groups of microorganisms including acid formers and hydrocarbon utilizers.[8] Like palm oil mill effluents, cassava wastewater is capable of producing lipolytic microbes including bacteria and fungi (mold and yeasts). The ability of these microbes to thrive in cassava wastewater could be associated with the availability of basic minerals in the wastewater. The microorganisms found in cassava wastewater could be converted into useful products of high demand. In the traditional setting in Nigeria, cassava wastewater is converted into starch. Cassava wastewater is rich in cellulose and hemicellulose. So microbes can mineralize the nutrients found in starches substrates such as cassava waste water with the action of additives to produced enzymes such as amylase, protease, cellulase, lipase, etc. Some of these enzymes such as cellulase,[9] amylase,[2,9,10] and protease[11-16] can be produced from some microorganisms. Adejuwon et al.[17] reported that *Candida albicans* produces an optimum protease at a temperature and pH of 30^0C and 7.0 respectively. Therefore, this paper focussed in assessing protease and amylase potentials of microbes isolated from cassava wastewater contaminated soil.

Materials and Methods

Data Source

The microbial isolates (*Escherichia coli, Staphylococcus aureus, Pseudomonas, Bacillus, Micrococcus, Proteus,* and *Enterobacter* species for bacteria, and *Saccharomyces cerevisiae, Aspergillus, Mucor, Penicillium* and *Rhizopus* species for fungi) used for this study were previously isolated from cassava wastewater contaminated soil by Izah and Aigberua.[18]

Amylase Assessment

Potatoes extract was incorporated into Nutrient Agar (for the determination of amylase producing bacteria) and Sabouraud Dextrose Agar (for the determination of amylase producing fungi) in the ratio of 40:60. The pure isolates were aseptically inoculated unto the respective agar plates and incubated for 24-48 hours (for bacteria) and 3-4 days (for Fungi/yeast) at 37°C. Then after, the agar plates were flooded with iodine solution, and a clear zone on the plates exposed to Gram's iodine is an indication of amylase producing ability of the isolates.[9,19-21]

Protease Assessment

Distilled water and liquid peak milk bought from a commercial shop in port Harcourt, Nigeria was incorporated into double strength media (Nutrient Agar used for protease producing bacteria, and Sabouraud Dextrose Agar for protease producing fungi) in the ratio of 35:65. Also, K_2HPO_4

2.0, peptone 5.0, gelatin 15.0 was added to the agar.[22,23] The pure were aseptically inoculated unto the respective agar plates for both groups of organisms and subsequently incubated at 37°C for 24-48 hours for bacteria and 3-4 days for fungi. The agar plates were flooded with mercury chloride.[22,23] A clear zone at the end of the incubation period around each isolate/colonies indicated protease production.[12,22,23] which was quantitatively expressed.

Results and Discussion

Table 1, shows the amylase and protease activities of microorganisms isolated from cassava wastewater contaminated soil. *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus* species (bacteria), and *Saccharomyces cerevisiae, Aspergillus, Mucor, Penicillium* and *Rhizopus* species (fungi) are both amylase and protease producers. While *Escherichia coli* and *Enterobacter* species did not produce amylase or protease. *Proteus* species showed a slight amylase ability and no protease potential. Also, *Micrococcus* showed protease activity and no amylase potential.

Table 1.Amylase and protease activity of microbes found in cassava wastewater contaminated soil

Organisms	Amylase activity	Protease activity
Bacteria		
Pseudomonas aeruginosa	+	+
Escherichia coli	-	-
Staphylococcus aureus	+	+
Micrococcus species	-	+
Proteus species	+	-
Enterobacter species	-	-
Bacillus species	+	+
Fungi (mould and yeast)		
Aspergillus species	+	+
Saccharomyces cerevisiae	+	+
Penicillium species	+	+
Mucor species	+	+
Rhizopus species	+	+

(+ = ability; and - = no ability)

The microbes found to possess amylase producing potentials in this study has some similarity with the findings of previous reports. For instance, Oboh[24] studied cassava mill effluents and reported that pure strains of *Saccharomyces cerevisiae* along with *Lactobacillus delbruckii* and *Lactobacillus coryniformis* for 72 hours are capable of producing amylase. Akpomie et al.[20] reported *Bacillus subtilis*, *Bacillus megaterium, Corynebacterium kutseri* and *Lactobacillus fermenti* as α-amylase producing microorganisms from cassava peels at an incubation temperature of 26–37°C. Similarly, Senthilkumar et al.[25] reported that *Bacillus* species have amylase activity using cassava as a substrate. The result presented here is also similar to other non-cassava substrates studies. Ohimain et al.[5] reported that *Staphylococcus aureus, Bacillus, Pseudomonas*,*Penicillium*, *Fusarium*, *Mucor*, *Candida* species and *Aspergillus niger* as amylase producing microorganisms from palm oil mill effluents. Kongkiattikajorn[26] reported that amylase and glucoamylase can be produced from *Saccharomyces diastaticus.* Ruban et al.[27] reported that *Bacillus subtilis* and *Aspergillus niger* isolated from soga effluents are capable of producing amylase. Sanni et al.[28] reported *Lactobacillus plantarum* and *Lactobacillus fermentum* as amylase producing microbes from different traditionally fermented foods in Nigeria. Ikram-Ul-Haq et al.[29] screened fungi isolates and reported that *Saccharomyces cerevisiae* and *Aspergillus niger* are α-amylase producers. Abu et al.[30] reported that a mixture of *Aspergillus niger* and *Saccharomyces cerevisiae* grew on sorghum pomace can hydrolyze its starch content to form amylase. Sugita et al.[31] studied amylase production from the intestinal microflora of freshwater fish and reported that *Pseudomonas, Chrostridium, Aeromonas, Bacteriodaceae* species can produce amylase. Oseni and Ekperigin[32] reported that *Streptococcus feacalis*, *Escherichia fruendi*, *Bacillus megatarium, Kurthia* species, *Erwinia amylovora*, *Lactobacillus acidophilus, Proteus vulgaris,*and *Proteus mirabilis* have *α-amylase* activity in an incubation temperature range of 30-60^{0}C. Alariya et al. [19] reported that *Pseudomonas fluorescens, Bacillus subtilus, Escherichia coli,*and *Serratia marscens* possesses amylase activity. *Escherichia coli* are not known to be amylase producers, but when an amylase producing gene from other microbes are inserted into the *Escherichia coli*, it could be transformed into an abundant amylase producer.[33]

The protease produced from microorganisms found in cassava wastewater contaminated soil is comparable to the report from other substrates. Authors have reported that different microbes possess protease activity depending on the minerals/ nutrients available. For instance, *Mucor circinelloides* produces protease using glucose as a substrate,[34] *Fusarium culmorum, F. avenaceum* and *F. oxysporum* using a mineral-protein medium and a cell wall medium.[35] Some other microbes that have been reported to possess protease activity include *Aspergillus,*[14] *Lactobacillus,*[15] *Bacillus, Staphylococcus*[16] and *Mucor* species.[13] Odu and Akujobi[36] reported that *Micrococcus luteus* and *Bacillus* species isolated from an abattoir environment produces protease which reaching optimum at 37^{0}C and 7.0 for temperature and pH, respectively. The authors further reported that *Bacillus, Pseudomonas, Halomonas, Arthrobacter and Serratia* as important protease producing

bacteria. Vermelho et al.[37] reported that *Pseudomonas aeruginosa, Micrococcus luteus* and *Serratia marcescens* have proteolytic activities. Chaturvedi et al.[38] reported that *Fusarium*, *Curvularia*, *Aspergillus* and *Mucor* species as good sources of extracellular protease production. Arsenijevic et al.[39] reported that *Candida* species have protease activity. Sharmin et al.[40] reported that *Lactobacillus* species isolated from the rumen have proteolytic properties producing at an optimum temperature, pH and incubation period of 37^0C, 8.0 and 2 days, respectively. Raja et al.[41] reported that *Penicillium* species have proteolytic activity on Skimmed Milk Agar Medium. Mishra[42] reported that *Lactobacillus delbrueckii* has been known for their protease producing activity and they further stated that it can mutate to give biochemical properties similar to *Neisseria flavescens.* Owoseni and Onilude[43] studied the protease production from *Enterobacter* species and *Escherichia coli* isolated from processed foods and reported that acid-stable protease, metalloprotease, and not serine-proteases reaches the maximum at 50^oC for both organisms. Microorganisms and its products particularly enzymes have been applied in different sectors. The extracellular enzyme activity of cassava wastewater could be attributed to the mineral and nutrient available in the effluents. Also, most of the microbes under study possess amylase and protease activity which is dependent on the incubation period and temperature, pH of the medium, source and biochemical characteristics of the isolates and choice of media used for investigation.

Conclusion

This study assessed the microbial isolates from cassava wastewater contaminated soil for the production of amylase and protease. The majority of the isolates including *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus* species (bacteria), and *Saccharomyces cerevisiae, Aspergillus, Mucor, Penicillium* and *Rhizopus* species (fungi) are amylase and protease producers. *Escherichia coli* and *Enterobacter* species cannot produce amylase and protease. While *Proteus* species showed slight amylase ability and no protease potential. Similarly, *Micrococcus* species only produced protease. As such, cassava wastewater contaminated soil could be a possible source of microbes for the production of extracellular enzymes needed by several industrial and biotechnology industries. By so doing, the attendant environmental impacts associated with the wastewater can be reduced.

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