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ISOLATION AND ACTIVITY OF ALPHA AMYLASE FROM SELECTED BACTERIA STRAINS IN THE FOREST SOIL

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ABSTRACT
Eight different bacteria species (*Streptococcus feacalis*, *Escherichia fruendi*, *Bacillus megatarium Kurthia spp*., *Erwinia amylovora*, *Lactobacillus acidophilus, Proteus vulgaris* and *Proteus mirabilis*) were isolated and identified from soil of Omo natural forest in Ogun State which was screened for the production of alpha amylase in liquid media using 2% starch as carbon source. All the strains of bacteria except *Bacillus megatarium* produced alpha amylase progressively from the second hour until optimally at the tenth and twelve hour of incubation respectively in the liquid medium. Among the organisms, *Erwinia amylovora*, *Lactobacillus acidophilus*, *Bacillus megatarium* and *Proteus mirabilis*) showed optimum activity at 30^0C while *Bacillus megatarium* showed activity maximally at 40^oC and (*Streptococcus feacalis*, *Escherichia fruendi*, and *Kurthia spp*.) showed enzyme activity optimally at 60^oC. The results obtained in this study revealed all the bacteria strains as promising sources of amylase for biotechnological applications, especially in starch industry for ethanol production, production of high-fructose corn syrup, production of shorter chains of sugars called oligosaccharides and production of dishwashing and de-starching detergents and host of other useful products.

KEYWORDS: forest-soil, bacteria-strains, alpha-amylase, optimum-activity, starch-industry.

INTRODUCTION

A vast number of organisms live in the soil (Schmidt *et.al.2002*); so great are micro floral numbers that they dominate the biomass in spite of the minute size of each organism (Russell, 1977). Together with the earthworms, the microflora monopolizes the metabolic activity in soils. It is estimated that 60 – 80% of the total soil metabolism is due to microflora. Not only do they destroy plant residues but they function in the digestive tracts of animals and eventually decompose the dead bodies of all organisms. Furthermore, soil humus is one of the significant end products of their activities (Brady, 1974). The ability of bacteria to degrade a variety of organic compounds is remarkable. Highly specialized groups of microorganisms play important roles in the mineralization of specific classes of organic compounds (Giri *et al.,* 2005). For example, the decomposition of cellulose, which is one of the most abundant constituents of plant tissues, is mainly brought about by aerobic bacteria that belong to the genus *Cytophaga.* This ability has also been utilized by humans in industry, waste processing, and *bioremediation* (Giri *et al.,* 2005). Starch degrading amylolytic enzymes are most important in the biotechnology industries with huge application in food, fermentation, textile and paper (Pandey *et al*., 2000). Amylases can be obtained from several sources such as plant, animal and microbes (Kathiresan and Manivannan, 2006). The microbial source of amylase is preferred to other sources because of its plasticity and vast availability. Microbial amylase has almost surpassed the synthetic sources in different industries (Pandey *et al*., 2000). Amylolytic enzymes are widely distributed in bacteria and fungi. They are categorized in to exo-acting, endo-acting and debranching enzymes. Among the amylases, β-amylase is exo-acting whereas α-amylase is endo-acting enzyme. α-amylases are hydrolytic enzymes that are widespread in nature, being found in animals, microorganisms and plants (Octávio *et al*., 2000). Amylases (α-amylase) are among the most important enzymes in present-day biotechnology. The enzyme has found numerous applications in commercial processes, including thinning and liquefaction of starch in alcohol, brewing and sugar industries (Riche`le, *et al.,* 1998). The present investigation therefore dealt with isolation and characterization of bacterial species, from soil samples collected from a forest reserve using physiological and biochemical features and hence determination of amylolytic activity and some kinetics of the enzyme.

MATERIALS AND METHODS

Isolation of bacteria

Amylolytic bacteria used in this study were isolated from soil of Omo natural forest reserve in Ogun State of Nigeria. 1.0g of the freshly collected soil was mixed with 9.0 mL of sterile distilled water in sterile test tube, serial dilutions were followed. 0.5mL of 10^{-3} dilution was pipetted into a sterile petri dish and overlaid with 20ml of nutrient agar. This was incubated at 37^0C for 24 hours. Many colonies were observed and each sub-cultured until a pure isolate was obtained. Pure isolates were maintained on nutrient agar slant and stored at 4^oC for further studies (Mishra, S. and Behera, N. (2008)).

Identification of Bacteria

Identification of isolates was performed by morphological observation, Gram staining, endospore staining, physiological and biochemical tests.

Preparation of Enzyme Solution

With the aid of a sterile cork borer, a 5mm disk from the advancing edge of 24 hours bacteria isolates were separately inoculated into the respective cultivation medium. Cultivation was carried out for 24 hours on mineral salt

Microbial enzymes Assays

0.5ml of enzyme solution was added to 0.5ml of substrate (1% soluble starch was prepared in 0.02M sodium phosphate of pH 6.9 containing 0.006M NaCl) and incubated at 25^0C for 3 minutes. 1ml of 3, 5-dinitrosalycylic acid (DNSA) was added. The mixture was then heated in water bath set at 100^0C for 5 minutes and cooled following which 10ml of distilled water was added. The color formed was read in a colorimeter at 540nm against a blank containing buffer solution without enzyme. A calibration curve was made with maltose (0.3 – 3.0µmoles) to convert colorimeter readings to unit of activity (Bernfeld, 1951).

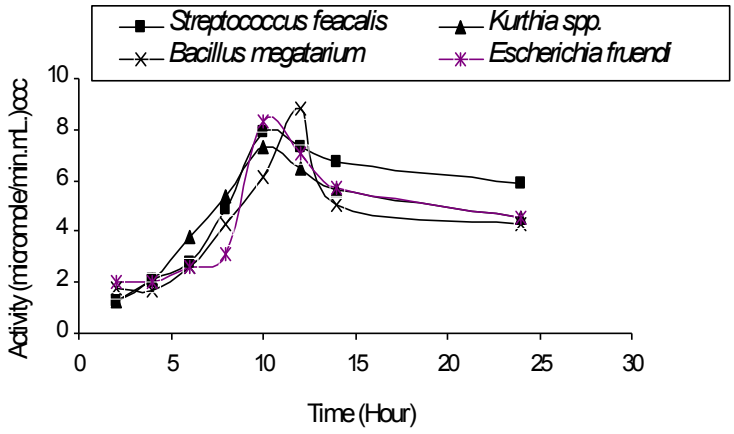

FIGURE 1: α-amylase activity produced by bacteria isolates in bacteria culture medium

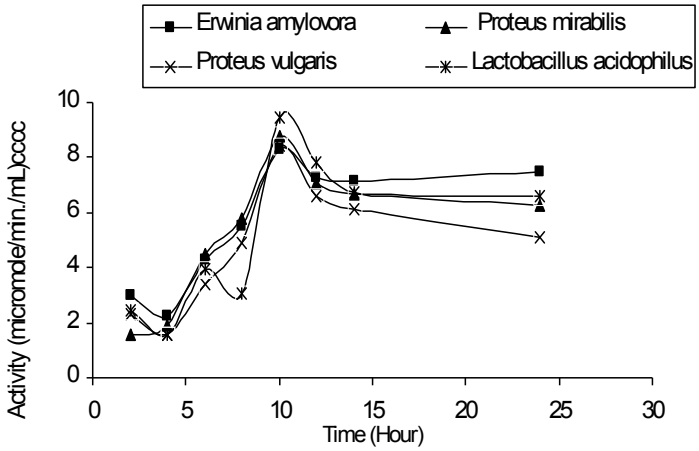

FIGURE 2: α-amylase activity produced by bacteria isolates in bacteria culture medium

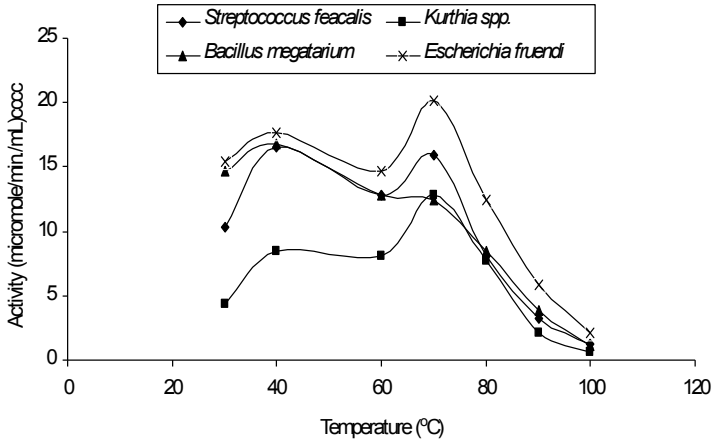

FIGURE 3: Effect of Temperature on α-amylase activity produced by bacteria isolates in bacteria culture medium

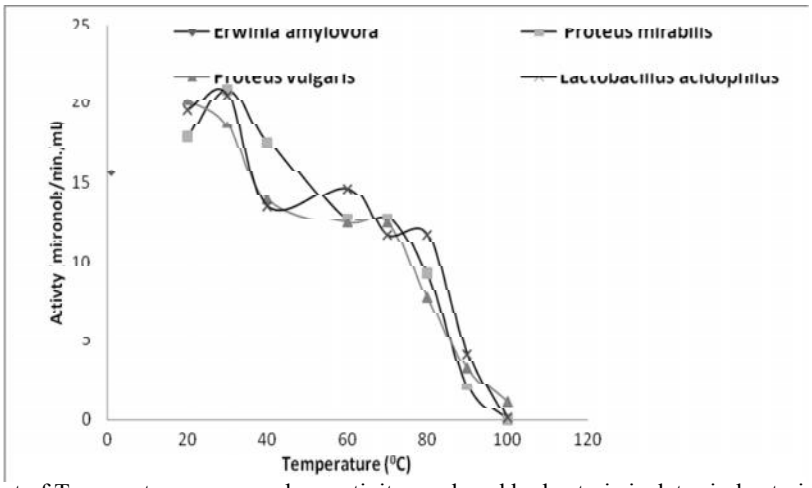

FIGURE 4: Effect of Temperature on α-amylase activity produced by bacteria isolates in bacteria culture medium

DISCUSSION

In the present investigation a pure strains of *Streptococcus feacalis*, *Escherichia fruendi*, *Bacillus megatarium, Kurthia spp*., *Erwinia amylovora*, *Lactobacillus acidophilus, Proteus vulgaris* and *Proteus mirabilis* were isolated from natural forest soil. The natural forest soil mostly consists of different types of broken down organic molecules including starchy materials and bacteria isolated from such places may have better potential to produce enzyme under adverse condition.

From Figures 1 and 2, all the organisms produced alpha amylase activity at 10th hour of culture except *Bacillus megatarium* that produced the enzyme at hour 12. The culture medium with 2% starch used in this work was found to be optimal for the enzyme cultivation and compared favourably with the work of Mishra and Behera (2008). Three of the bacteria (*Streptococcus feacalis*, *Escherichia fruendi,* and *Kurthia spp.* produced α-amylase optimally at 70^0C as *Bacillus megatarium* produced at 40^0C (Fig. 3), while *Erwinia amylovora*, *Proteus vulgaris*, *Lactobacillus acidophilus* and *Proteus mirabilis* produced maximally at 30^0C (Fig. 4). The incubation period of between 10 and 12 hours was observed to be the optimum time for all the bacteria studied. This is in agreement with the observations of other investigator Aiyer (2004), where he observed increased incubation period decreased amylase activity. The bacteria medium used in this work promoted the early production of the enzyme as it has been argued by Kathiresan and Manivannan (2006) that optimization of growth condition is a prime step in using microorganisms in fermentation technology. It was also reported in this work that the optimum temperature for optimum enzyme activity was in the range of 30-70^0C depending on the organisms. This observation was also corroborated by Mishra and Behera (2008) who reported a temperature range of 50-70^0C for a specie of *Bacillus.* All the organisms lost activity progressively after 50^0C until total lost at 100^0C, this observation was similar to that obtained by Saliu, (2009) who reported only 60% of the activity remained after heat treatment at 50ºC, 10% at 60ºC and no activity at 70ºC for a yellow pigmented bacterium isolated from cassava wastes obtained from a dumpsite near a gari processing factory in Ibadan, Nigeria.

CONCLUSION

This study has shown that these Gram negative, rod shaped, entire, rough and rhizoid edged bacteria are able to synthesize endo-acting amylase that is evidenced in the hydrolysis of starch and starchy materials from any source which is very important in biotechnology especially as regard production of enzymes using simple and cost effective materials. A look into the use of these bacteria that yield enzymes of different qualities may be of value in the modern day biotechnology and industries.

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