American Journal of BioScience
2020; 8(2): 41-44
http://www.sciencepublishinggroup.com/j/ajbio
doi: 10.11648/j.ajbio.20200802.13
ISSN: 2330-0159 (Print); ISSN: 2330-0167 (Online)

Production of Amylase from Bacteria Isolated from Tannery Effluent

Oyeleke Solomon Bankole[1, *], Erena Nuhu Bako[2], Manga Shuaibu Bala[3], Manga Sahabi[4]

[1]Department of Microbiology, Federal University of Technology, Minna, Nigeria

[2]Integrated Science Department, College of Education, Minna, Nigeria

[3]Department of Microbiology, Usumanu Danfodiyo University, Sokoto, Nigeria

[4]Department of Biological Sciences, Kebbi State University of Technology, Alero, Nigeria

Email address:

profoyeleke@gmail.com (O. S. Bankole), erenanuhu@gmail.com (E. N. Bako), sbmanga@hotmail.com (M. S. Bala), ssmanga2000@yahoo.com (M. Sahabi)

[*]Corresponding author

To cite this article:

Oyeleke Solomon Bankole, Erena Nuhu Bako, Manga Shuaibu Bala, Manga Sahabi. Production of Amylase from Bacteria Isolated from Tannery Effluent. American Journal of BioScience. Vol. 8, No. 2, 2020, pp. 41-44. doi: 10.11648/j.ajbio.20200802.13

Received: November 6, 2019; **Accepted**: November 23, 2019; **Published**: May 18, 2020

Abstract: Bacteria isolated from tannery effluent collected from Erena local tannery in Niger State were examined for amylase production. Two of the isolates, *Bacillus subtilis* and *Psuedomonas aeruginosa* demonstrated ability of amylase production, where *Bacillus subtilis* has it highest amylase activity of 2.1mg/ml at 18hr, while *Psuedomonas aerugionsa* has highest activity of 1.7mg/ml at the same period. The optimum temperature and pH were also determined, *Bacillus subtilis* has highest amylase activity of 2.5mg/ml at 60°C, while *Psuedomonas aeruginosa* has highest activity of 2.3mg/ml at 80°C. The optimum pH for amylase produced by *Bacillus subtilis* and *Psuedomonas aeruginosa* at pH 7 were found to be 2.8mg/ml and 2.7mg/ml respectively. *Streptococcus faecalis* was not able to produce amylase. The research recommended that, production of the enzymes (amylase) from the two bacteria (*Pseudomonas aeruginosa* and *Bacillus subtilis*) will pave way for industrialization and reduced cost of production and procurement of this enzymes. The production of amylase by *Psuedomonas aeruginosa* and *Bacillus subtilis* will also go a long way to conserve our hard earn foreign exchange thereby enhancing the economy, if these orgasms are fully exploited for the production of the enzyme, as well as utilizing the waste effluent that can pollute the environment to a beneficial aspect.

Keywords: Tannery, Effluents, Amylase, Bacteria, Isolates

1. Introduction

Amylase is an enzyme that breaks starch down into sugar. Plants and some bacteria has the potential of producing amylase. Amylse was first discovered in 1946, where amylase was found to be produced by *Bacillus polymyxia* and later by another *Bacillus cereus* [9]. As diastase, amylase was the first enzyme to be discovered and isolated. Specific amylase proteins are designed by different Greek later. Amylase is the most important enzymes used in biotechnology [5].

Members of the genus Bacillus are heterogeneous and they are very versatile in their adaptability to the environment. There are various factors that influence the nature of their metabolic processes and enzymes produced [1, 9]. *Bacillus specie* produce a large variety of extracellular enzymes, such as amylases which have significant industrial importance [6]. In the same vein, bacterial enzymes are known to possess more thermo stability than fungal amylases [14].

Numerous microorganisms like *Saccharomycopsis capsularia, Bacillus coagulans, Bacillus specie* Hop-40 and *Bacillus megatarium* 16M have been used for alpha-amylase production using solid state fermentation (SSF) [10]. Studies by [13, 14] reported the production of thermos table alpha-amylase by *Bacillus lincheniformis* and *Bacillus* megaterium at highest frequencies. *Aspergillus specie*, *Bacillus specie* and *Rhizopus specie* has been reported to be potential producers of alpha amylase. A number of substrate has been employed

for the production of microbial enzymes. Some of the substrates that have been used include sugar cane bagas, rice straw, rice husk, banana peels cassava peels, palm oil mills waste, peanut meal, coconut oil cake waste, cassava flour, corn flour, steamed rice, and starch [9]. Tannery effluent can also be a potential source of microbial enzyme producer because of its content of natural proteins [8, 3].

The objective of the research to produce amylase enzymes from microorganisms isolated Tannery effluent to determine their optimum condition for growth and production.

2. Material and Method

2.1. Isolation and Identification

One litre of tannery effluents was collected in a sterile bottle at Erena local tannery industry, in Shiroro Local Government Area of Niger State, Nigeria for preparation of initial cultures. These were sub-cultured to obtain pure isolates of bacterial species as described by [11]. The isolated bacteria were identified based on cellular morphology, growth conditions, gram stain, and motility and biochemical tests.

2.2. Screening for Amylolytic Activity

Using the method of [4], the amylolytic ability of the isolated organisms was determined. This was done by introducing 1 ml of cells of each bacterial isolate into a 2mm hole aseptically on nutrient agar medium which was fortified with 1%(w/v) starch (i.e 2.5g of starch). The agar plate was incubated at 37°C for 24hours. The incubated plates were then flooded with Dinitrosalicyclic acid (DNSA) Reagent, and later transferred into an oven and incubated at 45°C for 30 mins. Haloes produced around the zone of inoculation of the isolates were measured using a graduated ruler. Theses represented the amylolytic activity of the strains.

2.3. Amylase Production

The culture medium were prepared by weighing the following medium compositions in grams per litre a; soluble starch, 1.0; bacteriological peptone, 6.0; ($MgSO_4.7H_2$), 0.5. KC1, 0.5. served as inoculums media for all the experiments. This was sterilized at 121°C for 15 min. Prepared media plates were inoculated with the bacterial isolates and were incubated for 24 hours. The selected strains of isolates were propagated at 37°C for 24 hours in 50ml of 8%(w/v) of starch medium placed in 250ml Erlenmeyer flasks and placed in a shaker incubator operated at 120rpm at 30°C.

The extracellular enzyme solutions were obtained by centrifugation at 5000rpm for 20 minutes using a high speed centrifuge. The supernatant obtained was collected and used as enzyme sources as well as enzyme assays.

2.4. Determination of Amylase Activity

Amylase activity was assayed by pipetting 0.5 ml of culture extract "enzyme" into test tubes and 1ml of 1% soluble starch in citrate phosphate buffer having a pH of 6.4. The reducing sugars liberated were estimated by the 3, 5–dinitrosalicyclic acid (DNSA) method. The reaction mixture was incubated in a water bath at 40°C for 30 minutes. A blank consisting of 1ml of soluble starch in citrate–phosphate buffer (pH 6.4) was also incubated in a water both at the some temperature and time with the other test tubes. The reaction was terminated by adding 1ml of DNSA reagent in each test tube and then immersing the tubes in a boiling water bath for 5 minutes after which they were allowed to cool and 5ml of distilled water was added. The absorbance for all the test tubes was measured at 540nm with spectrophotometer.

2.5. Determination of Optimum Temperature

The optimal temperature for activity was determined by assaying activity of amylase produced by *Bacillus subtilis* and *Psuedomonas aeruginosa* at different temperature ranges of 30, 40, 50 60, 70, 80 90 and 100°C. Thermo stability of the enzyme was done by maintaining the enzyme solution in a water bath at theses different temperature for 30 minutes. DNSA was added and the absorbance was taken on the spectrophotometer at 540nm. This was done according to the method describe by [4].

2.6. Determination of Optimum pH for Amylase Production

The reaction mixtures of 72 containing enzymes from *Bacillus subtilis* and *Psuedomonas aeruginosa* were prepared as earlier described and the optimum pH for enzyme (amylase) activity as was examined by running the assay activity between pH ranges of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10. The enzyme activity for each pH was determined using the method of [4]. The pH stability was determined by incubating the partially purified enzyme in water bath at 70°C and the residual activity was then measured by adding the DNSA reagent and taking absorbance for each pH using spectrophotometer at 540nm. Adjustments of the pH was done by addition of hydrochloric acid (0.1N) and 0.1N sodium hydroxide to achieve acidity and alkalinity respectively.

3. Result and Discussion

Three (3) bacterial isolates were obtained from tannery effluent and identified as *Streptococcus faecalis,, Bacillus Subtilis* and *Psuedomonas aeruginosa.* Two (2) of the isolates (*Bacillus subtilis* and *Ppsuedomonas aeruginosa)* were able to produce amylase.

The activity of amylase production revealed that the highest enzyme production period for *Bacillus subtilis* was at 18 hours (figure 1) with maximum activity of 2.1 mg/ml, while that of *Psuedomonas aeruginosa at* 18 hours also with activity of 1.7mg/ml. Activity was lost gradually after this hour, this is said to be the stationary phase whee there is no cell growth and the viable cells remain constant. At the 24 hours, viable cells began to decline, thus resulting in a death

phase. Similar reports were observed by [12]. and [14]. regarding the use of *Bacillus species. Bacillus megaterium* isolated from cassave processing waste and local yam peel waste dumpsite respectively for the production of amylase enzyme.

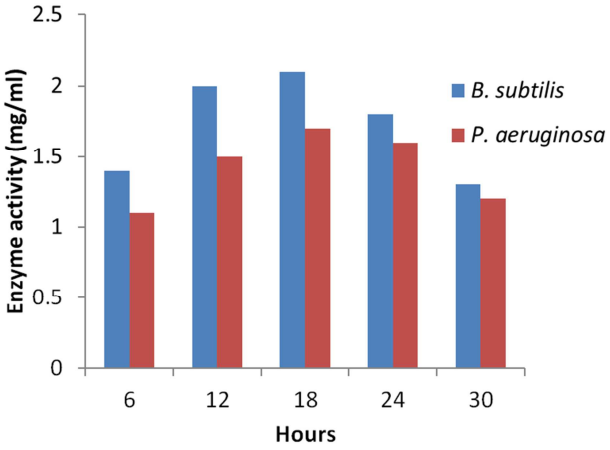

Figure 1. *Amylase activity of Bacillus subtilis and Psuedomonas aeruginosa.*

Increase in incubation period resulted in decrease in the production of enzyme by *Bacillus subtilis.* This may be due to the fact that, after maximum production of enzyme (maximum incubation time), there was production of other metoblites. These metabolites inhibited the growth of the organisms and hence, enzyme formation (2., 9.). And a depletion of nutrients [14].

Optimum temperature of amylase produced by *Bacillus subtilis* was at 60°C, and 80°C for *Psuedomonas aeruginosa (*Figure 3). The two isolates had amylase activity ranging from 0.6–2.5mg/ml/sec. This result agrees with [13], who recorded optimum amylase activity at 60°C by *Bacillus megaterium,* and also that of [7, 12]; and [13]., they reoirted that during isomerisation, temperature is preferably maintained within the range 20–90°C and the best activity of obtained with 50-75°C.

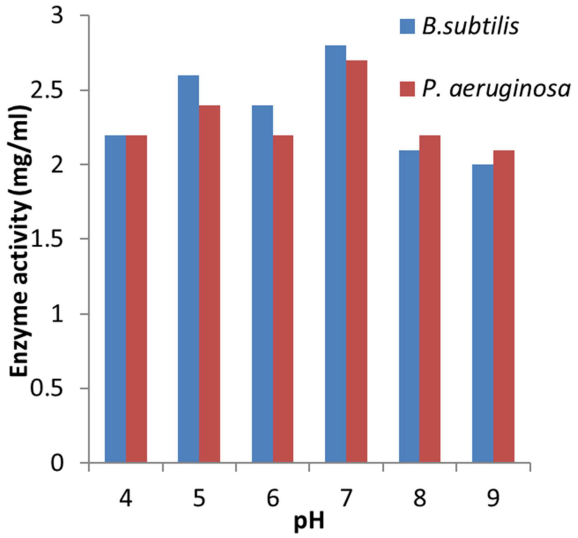

Figure 2. *Effect of pH on enzymatic activity of amylase produced by Bacillus subtilis and Psuedomonas aeruginosa.*

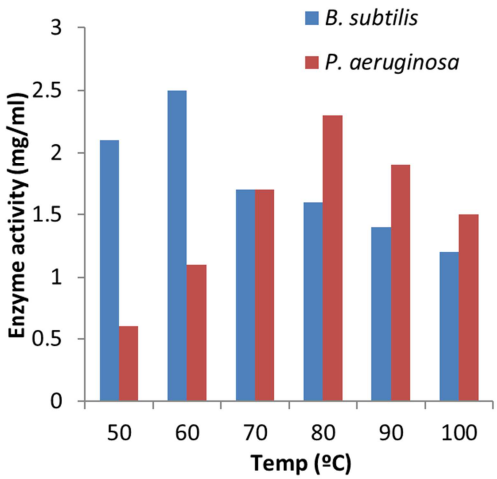

Figure 3. *Effect of temperature on amylase activity produced by Bacillus subtilis and Psuedomonas aeruginosa.*

Optimum pH of amylase produced by *Bacillus subtilis, Pseudomonas aeruginosa* was uniform for the two isolates in that all had their optimum activity at pH 7 which was then followed by a steady decrease in activity, though the was an increase in amylase activity by *Bacillus subtilis* at pH 5 (2.6mg/ml) this was flowing by a sharp decrease in amylase activity at pH 6 (2.4mg/ml and further increased amylase activity at pH 7 (2.8mg/ml), thuse the peak activity was recorded at pH 7. *Pseudomonas aeruginosa* had an amylase yield of 2.7 mg/ml at pH 7 (figure 2).

The enzyme stability trend, as reported in the present study, agrees with the behavior of amylases from *Bacillus species*, investigated by [6]. in which a soluble starch medium was used. This result agrees with [12]. who recorded optimum pH activities at 6.5 to 7.5. in the production of amylase, using *Bacillus subtilis.*

4. Conclusion

Two isolates (*Psuedomonas aeruginosa* and *Bacillus subtilis*) has demonstrate ability to produce amylase and the production of amylase by these microorganisms will go a long way to conserve our hard earn foreign exchange if these orgasms are fully exploited for the production of this enzyme. As well as utilizing the waste effluent to a beneficial aspect.

Acknowledgements

I wish to thank the laboratory technicians of Federal University of Technology Minna and those of the microbiological laboratory of Niger State Water Board for their assistance and contribution during the conduct of the research.

References

[1] Ajayi, A. O. and Fagade O. E. (2006) Growth pattern and structural nature of amylases produced by some *Bacillus species* in starchy substrate. *Africa journal of Biotechnology. 5 (5); 440-444.*

[2] Ali G. A. O. (1992). Formation of protease by *Aspergillums fumigates* and *Penicillium* spp. *Journal of King Saad University of science* 4 (2); 127.

[3] APHA (1995). Standard methods., standard method for the examination of water and wastewater. American public Health Association American water works Association and waters environment federation Eaton Ad, Clesceri LS, Greenberg AE.

[4] Bertrand T. F., Fredric T. and Robert, N. (2004), Production and partial characterization of a thermostable amylase from *Ascomycetes* yeast strain isolated from starchy soil. McGraw Hill Inc. York Pp 53-55.

[5] Burham, A., Nisa U., Gokhan C., Ashabil A., and Osmar G. (2003). Enzymatic properties of award thermostable thermophilic alkaline and chekato resistant amylase from an alkaphilic *Bacillus spp* isolate ANT-6. *Proceedings of Biochemistry*; (38) 1397-1403.

[6] Cordeiro, C. A. M. Martinas M. I. L. and Lucaino A. (2003). Production and properties of alpha amylase from thermophylic *Bacillus* specie. *Brazilian journal of microbiology* (933) 1-3.

[7] Daniel, R. M., Peterson M. E. and Dauson M. J. (2010). The molecular basis of the effect of temperature on enzyme activity. Journal of Biochemistry. 425 (2) 353-360.

[8] Frolund, B. Palmgren R., Keiding K. and Nielsen PH. (1995) Enzymatic activity in the activity in the activated sludge floe matrix. *Applied Microbial Biotechnology (43) 708–16.*

[9] Gupta A., Gupta V. K.., Modi D. R. and Yadava L. P. (1995). Production and characterization of *a-amylase from Aspergillus niger, Journal of Biotechnology 7: 551-556.*

[10] Modi, H. A. Patel K. C., and Ray R. M. (1994). In solid state fermentation (ed pandey wiley Eastern publishes, New Delhi pp 137-141.

[11] Oyeleke S. B and Manga S. B. (2008) Essential of *laboratory practicals in Microbiology* (1st ed Tobest publisher, Niger State pp 36-58.

[12] Oyeleke S. B. and Oduwale A. A. (2009) Production of Amylase by bacteria isolated form a cassava waste dumpsite in Minna, Niger, Nigeria. *Africa Journal of Microbial Research* 3 (4) 143-146.

[13] Oyeleke S. B., Auta S. H, and Egwin E. C. (2010). Production and characterization of amylase produced by *Bacillus megaterium,* from a Local yam peel dumpsite in Minna, Niger State. *Journal of microbiology and Antimicrobials* 2 (7) Pp 88-92.

[14] Oyeleke S. B., Auta S. H and Egwim E. C. (2011). Screening of *Aspergillus flavus* and *Aspergillus fumigatus* strains for extracellular protease enzyme production. *journal of Microbiology and Antimicrobials* 2 (7) Pp 83-87.