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# SCREENING AND PRODUCTION OF ALPHA GLYCOSIDE HYDROLASES (GH13 Family) FROM NEWLY ISOLATED BACTERIAL STRAIN

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**Abstract**: One of the most common enzymes used in industrial targets is glycoside hydrolase. The starch molecules are hydrolyzed by similar enzymes to form polymers made up of glucose units. α-glycoside hydrolases may find use in a wide range of industrial processes, such as those in the food, fermentation, and pharmaceutical sectors. A key component of many effectively necessary crops, including wheat, rice, maize, tapioca, and potatoes, starch is also a crucial component of the diet. Starch-transforming enzymes are used to produce glucose and fructose syrups, maltodextrin, or improved starches. This study isolated a new strain of α-glycoside hydrolases from rice field soil. The focus was on improving fermentation conditions to increase the production of α-glycoside hydrolases. After a 24-hour incubation period, a customized artificial medium was created that achieved the desired bacterial growth and enzyme yield. The ideal conditions for the production of α-amylase were found to be 40°C and pH 7.00. The potential of bacterial strains as a reliable source of α-glycoside hydrolases is highlighted in this work, along with the importance of maximizing growth conditions and medium conformation to achieve efficient enzyme production.

**Key words**: glycoside hydrolases, bacteria, Biocatalyst, α-amylase, microorganisms

## INTRODUCTION

The other form of Glucosidase Hydrolase which is more popular and commonly used is alpha glycoside hydrolase. Alpha glycoside hydrolase is one of the most important and widely used industrial enzymes. They are hydrolytic enzymes that break down starch and other polysaccharides by binding to certain glycosidic bonds, and resulting in the formation of dextrins and glucose. Alpha glycoside hydrolases are used in food, textile, paper and brewing industries (Gupta *et al.*, 2003; Khanra *et al*, 2015). Alpha glycoside hydrolases (E.C 3.2.1.1) works on starch, glycogen and other polysaccharides in a random manner and finally gives limit dextrin, maltotriose, maltose and glucose (Takeshita and Hehre, 1975; Shaw *et al.*, 1995). Alpha glycoside hydrolases is secreted by animals, plants and microorganisms, however the microbial enzymes are more widely used in the industrial production of alpha glycoside hydrolases because of the lower cost and ease of production with less space and time requirements (Burhan *et al.*, 2003. The α-glycoside hydrolases produce by bacteria and fungi are particularly interesting for industrial processes. Aspergillus fungi and Bacillus bacteria are frequently employed in the commercial synthesis of thermostable α-glycoside hydrolases (Shi *et al.*, 2022; Campbell, 1954). Fermentation is used in large bioreactors to produce α-glycoside hydrolases for commercial use. During fermentation, a number of variables should be optimised, including nutrient concentration, medium pH and temperature, level of aeration, and contamination control. According to reports, microbial α-glycoside hydrolases are inducible enzymes whose synthesis is increased in response to an inducer, which is typically a substrate unique to the inducible enzyme. Fermentation media should contain a substrate with α-1,4 glucosidic linkages, such as maltose, dextrin, and starch, in order for microbial cells to express α-glycoside hydrolases (Gupta *et al.*, 2003; Hillier *et al.*, 1997). Microbes could produce α-glycoside hydrolases through solid state fermentation or submerged fermentation. Because solid state fermentation is similar to natural microbiological processes like composting and ensiling, it is typically used to produce α-glycoside hydrolases. [8,9]) and to achieve economy in enzyme production (Hewitt and Solomons, 1996). α-glycoside hydrolases are among the most crucial hydrolysing enzymes in all starch-based industries, particularly thermostable microbial α-glycoside hydrolases. They have a vast area of study and practical uses in the paper, textile, food, and detergent industries. Additionally, advancements in genetic engineering have made enzyme yields using enzymes with specific characteristics, like increased consistency and application methods like induced mutations with chemical agents (Haq *et al*., 2010). These methods enable the creation of in industrial settings. The ongoing need for α -glycoside hydrolases has made this enzyme extremely significant, contributing around 30% of global commercial enzyme. The global α-glycoside hydrolases (α-amylase) market was estimated at $3.12 billion in 2024 and is projected to reach $5.75 billion by 2033, growing at a compound annual growth rate (CAGR) of approximately 7.29% during the forecast period of 2026-2033.

## MATERIALS AND METHODS

***Sample collection***

There are several sources of α-glycoside hydrolases in the environment, including plant, animal, and microbial sources. Since rice and potatoes are the sources of starch, soil samples were taken from rice and potato growing fields for the α--glycoside hydrolases production. The soil samples that contained the bacteria were used for production of enzyme.

***Serial dilution***

In order to achieve a fine growth in the specified medium, the step lowers the number of bacteria cells and gives the sample a desirable range of bacteria. Fill the sterile bottle with 10 ml of the distilled water that was measured in the graduated cylinder. After weighing a 1.0 g sample of soil, mix it with sterile distilled water.

To ensure the solution is well mixed, tightly cap the bottle and shake it. The sterile tubes should be labelled 10-1, 10-2, 10-2, 10-3, and up to 10-9. Fill each tube with 9.0 ml of distilled water. With a fresh pipette close to the flame, transfer 1.0 ml of the solution from the bottle to the 10-1 label. Cover the tube and gently swirl it until the solution is thoroughly combined. Using a fresh pipette, move 1.0 ml of the solution from the 10-1 test tube to the 10-2 tube. To mix, cap the 10-2 tube and swirl. Transfer the solution from the 10-2 tube to the 10-3 tube, then from the 10-3 tube to the 10-4 tube, and so on, until 10-9.

***Strain isolation***

In order to isolate strains, we used the spread plate technique, which involves placing the fewest bacterial cells in a petri plate with appropriate media using the last dilution tube (10-9).

***Media preparation***

Add 50.0 ml of distilled water, 1.0 g of nutrient broth, and 0.5 gm of starch powder in conical flask. Once the starch and nutritional broth have been dissolved, add enough distilled water to reach 100 ml and p H was adjusted to 7.0. Add 2.20 gm of agar-agar to the solution. Sterilize the medium at 121°C using autoclave. After that, add the sterilized media to the petri dishes and wait for it to solidify.

***Procedure***

Transfer 0.1 ml of the inoculation tube to the sterile petri plate with the agar media close to the flame. Use ethyl alcohol to rinse the glass spreader. To light the alcohol, pass the spreader by the Bunsen burner. Use the spreader once the alcohol has been removed. Using a spreader, evenly distribute the 0.1 ml solution across the media surface close to the flame, minimizing the possibility of contamination. The petri plate should be incubated for 24 hours. After a day, check the bacterial growth.

***Streak plate method***

Organisms (primarily bacteria) from mixed populations are separated into pure cultures using the streak plate technique. The inoculum is streaked over the agar surface to "thin out" the bacteria. As the original sample is streaked over successive quadrants to dilute it, the number of organisms decreases. The third or fourth quadrant usually transfers only a few organisms, producing unique colony-forming units.

***Effect of substrate concentration on α-glycoside hydrolases production***

Substrate concentration play an important role in enzyme production. In the fermentation media, varying concentrations of starch (0.25% to 2.0%) were added.

***Effect of temperature on α-glycoside hydrolases production***

Bacteria were added to the selected medium, and it was then incubated at various temperatures (30°C and 40°C). 50°C and 60°C for a full day.

***Effect of pH on α-glycoside hydrolases production***

Media with varying pH values between 5-8 were prepared in order to determine the ideal pH for maximum enzyme production, and culture were then incubated for 24 hours at 40°C.

***Effect of time on α-glycoside hydrolases production***

The bacterial strain was inoculated into the chosen medium and incubated for varied lengths of time in order to identify the peak of cellular growth and the production of α-glycoside hydrolases.

***Enzyme Assay***

Glucoside hydrolase activity was determined by quantification of the reducing sugar released by hydrolysis of starch using maltose as a standard (Miller, 1959). In both the test and control tubes, add 1.0 ml enzyme. Add 0.5 ml of NaOH in control marked tube to denatured the enzyme. Add 1.0 ml of the substrate (0.1gm starch in phosphate buffer, pH 7.0) in both the test and control tubes and kept for 15 minutes at 40°C. Following the incubation, add 0.5 ml NaOH only to the test marked tubes to stop enzyme substrate reaction. Take 1.0 ml from the test and control tubes into separate empty tubes. Distilled water was used to prepare Blank reagent. Add 1.0 ml Nelson reagent to all tubes and placed in heating water bath for 20 minutes. After, cooling, add 1.0 ml arsinomolybedate to all the tubes and read at 578 nm against water blank.

***Enzyme Units***:

A α-glycoside hydrolases unit is defined as **"the *amount of enzyme that liberates 1.0 µmol of reducing sugar from the substrate in one minute at 40 °C from soluble starch"*.**

## RESULTS AND DISCUSSION

There are two kinds of colonies identified as producers and non-producers, and they differ in size, shape, and structure.

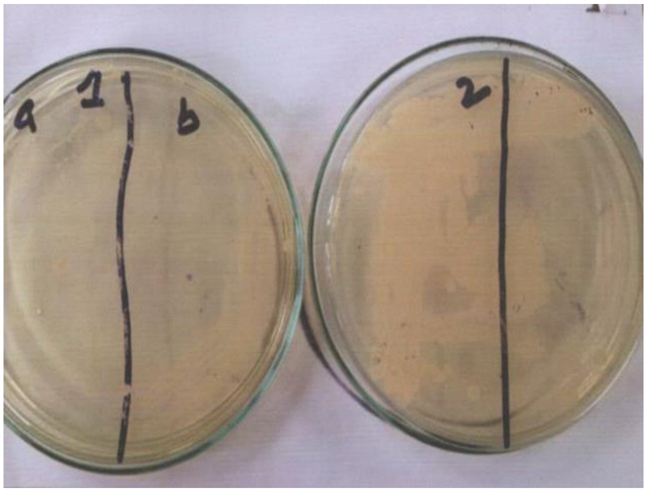


**Fig 1: (la) Producers and Non-Producers. (1b) The shiny, pointed non-producing bacteria. (2) Manufacturer of irregularly shaped *α*-glycoside hydrolases**

***Culture isolation for the screening of α-glycoside hydrolases***

To identify the ***α***--glycoside hydrolases producing bacteria in the mix culture bacterial media react with iodine solution. As iodine changes from dark purple to yellow color due to hydrolisation, indicates the present of α-glycoside hydrolases in that region.

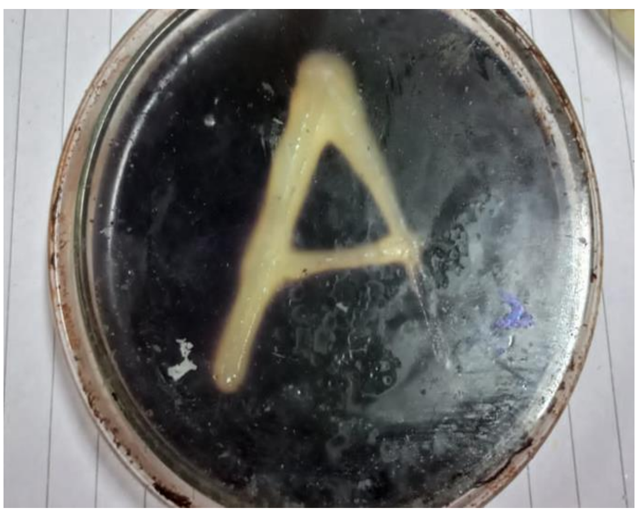

**Fig 2**: ***α*-glycoside hydrolases screening by iodine solution**

***W-streaking of selected bacterial strains***

The W-streaking is the process by which the cultured bacteria streak across the agar medium in a W shape. Iodine production indicates the region surrounding the streak where α-glycoside hydrolases are produced.


**Fig 3: Showing bacterial growth and α-glycoside hydrolases producing zones**

***Effect of starch concentration on α-glycoside hydrolases production***

Different carbon sources and their concentration in the fermentation medium affect the production of enzymes (Welker and Campbell, 1963). Numerous researchers have noted that one of the main carbon sources used in the fermentation process to produce amylases is starch (Bajpai and Bajpai, 1997, Bano *et al*., 2011). The maximum production of α-glycoside hydrolases was supported by starch at a concentration of 1.0 gm% in our study; as the concentration rose, the production of enzymes decreased (Figure 4). The formation of glucose during catabolic repression may have been the cause, and this glucose may have had a detrimental effect on the expression of the α-glycoside hydrolase gene. Similar results were reported by Haseltine et al. (1996), who found that the production of amylase was suppressed by the glucose generated during the fermentation process by *Archaeon Sulfolobus solfataricus*

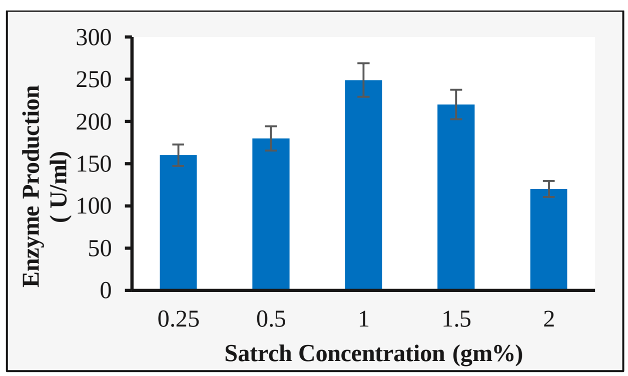


**Fig 4: Effect of starch concentration on *α*-glycoside hydrolases production**

***Effect of temperature on α-glycoside hydrolases production***

Temperature always plays a key role on the production of α-glycoside hydrolases during fermentation process. According to the current study, the bacterial strain's α-glycoside hydrolase production peaked at 40 °C and then declined as the temperature rose to 60 °C (Figure 5). According to some researchers, the amount of dissolved oxygen in the fermentation medium has an impact on the production of the enzyme amylase by various Bacillus species (Qader *et al.*, 2006). High temperatures cause oxygen to become less soluble, which limits the amount of oxygen available for the fermentation medium's α-amylase production (Campbell and Pace, 1968). It has been reported that difference in the level of oxygen in the medium induced changes in the surface protein layer (S-layer) of bacterial cell membrane (Sara *et al.*, 1996) which is involve in the release of extracellular of enzyme.

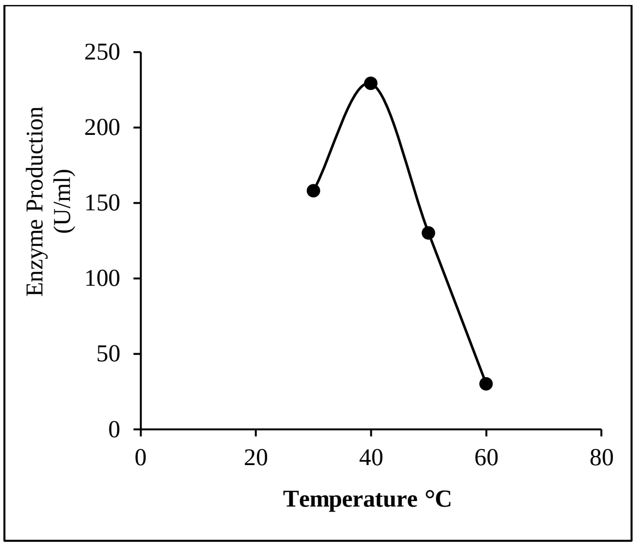


**Fig 5: Effect of temperature on *α*-glycoside hydrolases production**

***Effect of pH on α-glycoside hydrolases production***

Role of fermentation medium pH is very important for enzyme production. In the current investigation, the highest level of enzyme production was attained when the medium's initial pH was maintained at 7.00 prior to sterilization (Figure 6); at pH 5.00 and pH 8.00, respectively, production was observed at 30% and 25%, in relation to the optimum pH. It was reported that when the media's pH is maintained between 6.0 and 9.0, the majority of commercial Bacillus strains produce α-amylase (Gupta et al., 2003, Burhan et al., 2003). The altered morphology of the bacterial cell wall may be the cause of the decreased enzyme production below and above the ideal pH. According to a report, the medium pH has an impact on the composition of microorganisms' cell walls (Ellwood and Tempest, 1972). Growth parameters may change as a result of these changes in the characteristics of the cell wall and plasma membrane, and the medium's pH may no longer be appropriate for the organism's growth (Stutzenberger and Jenkins, 1995). This could be the cause of the current study's lower *α*-glycoside hydrolases production at pH 5.0 and 9.0.

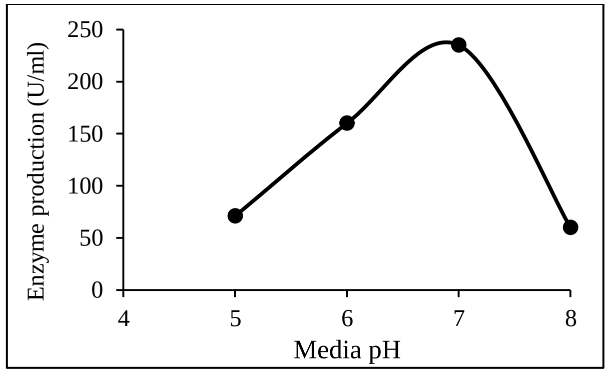


**Fig 6: Effect of media pH on *α*-glycoside hydrolases production**

***Effect of time on α-glycoside hydrolases production***

For optimal enzyme production, the bacterial culture was cultivated in the fermentation medium for varying durations, ranging from 06 to 72 hours (Figure 7). Cell growth and enzyme production were found to be linearly related, with the highest levels of α-glycoside hydrolases and cell growth occurring after 24 hours. Additionally, it was discovered that cellular growth began to slow down after 24 hours, which in turn led to a decrease in the production of enzymes. This could be because bacteria are susceptible to metabolite repressions that occur during growth (Cordeiro *et al*. 2002). Researchers also found that bacterial strains needed the same amount of time to grow to their maximum capacity and produce α-glycoside hydrolases (Bajpai and Bajpai, 1989; Saito and Yamamoto, 1975).

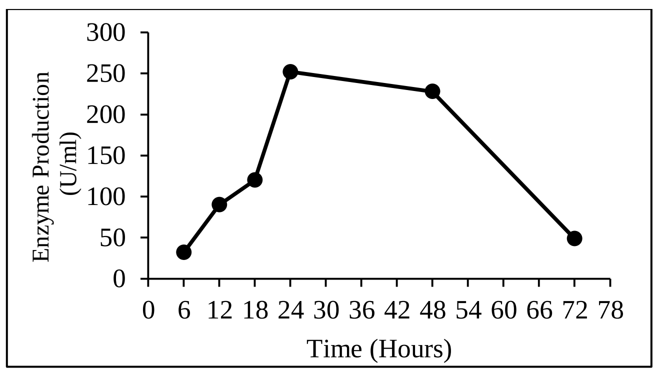


**Fig 7: Effect of time on *α*-glycoside hydrolases production**

## CONCLUSIONS

This study is significant because it suggests that our findings are consistent with previous reports in which numerous scientists isolated bacteria that produce α-glycoside hydrolases(α-amylase) from soil samples, identified different species, and carried out biochemical experiments. With a variety of microbial sources available for an efficient enzyme production, the use of α-glycoside hydrolases (α-amylase) in starch-based industries has been widespread for some time. However, only a small number of bacterial and fungal strains satisfy the requirements for production on a commercial scale. However, the primary focus of all research has always been on microorganisms that can produce α-glycoside hydrolases. Additionally, a number of researchers have documented successful outcomes in the synthesis of α-glycoside hydrolases, which has made it easier to use in the clinical and pharmaceutical fields where high-purity enzymes are now crucial. Our results showed that the optimal starch concentration for α-glycoside hydrolase production was 1.0 gm%, which was utilized as a carbon source. Following tests, the highest level of enzyme production was observed at pH 7.0. Since the isolated strain needed 24 hours to grow and produce its enzymes to their full potential, 40°C is the ideal temperature.

***Conflict of interest***

Authors declare no conflict of interest.

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