Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [3] 2022: 160-165

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Isolation and Optimization of Alkaline Amylase from Marine Bacteria

Vedant Vala [1], Prasad Andhare[3], Indrani Bhattacharya[2], Anjali Thakur[2], Dhwani Upadhyay[2]*
[1-2]Parul Institute of Applied Science, Parul University, Post Limda, Waghodiya, Gujarat
[3]: Assistant Professor, Biological Sciences, PDPIAS, Charotar University of Science and Technology, Changa, Anand, Gujarat.
*Corresponding Author: Dr. Dhwani Upadhyay;
E-Mail: dhwaniupadhyay82123@paruluniversity.ac.in

ABSTRACT
Amylase is a macromolecular catalase enzyme that breaks down starch and similar polysaccharides into simple sugars. Amylase is utilized in breweries, foods, detergents, starch processing, textiles, pharmaceuticals, and a range of other sectors. Amylase reacts with starch, hydrolyzing α -1, 4 glyosidic bonds to produce the short polymer dextrin, followed by maltose, and lastly glucose. Amylase is divided into three categories: amylase, amylase, and amylase. In the present study, the alkaliphilic amylase producing bacterium was isolated from the marine water sample. The isolation was carried out on starch agar plate followed by quantification using DNSA method. The isolate showed 161.5U/mL enzyme activity in crude enzyme sample. The bacterium was subjected to morphological and biochemical test further. The bacterial growth medium optimization studies revealed that the isolate was having the highest enzyme activity at pH 10, temperature 37°C and 3% substrate (starch) concentration. Ammonium sulphate precipitation and dialysis were used to partly purify the enzyme. The enzyme that has been purified showed the enzyme activity 170U/ml.
Keyword :- Enzyme, Alkaliphilic amylase , alkaliphilic bacteria , production , optimization , industries.

Received 06.08.2022 Revised 17.09.2022 Accepted 28.10.2022

INTRODUCTION

Enzyme is a colloidal, high molecular weight, non-dialyzable, denaturable, and structurally diverse protein. Enzymes increase the rate of reaction in a process (1). Initially, enzymes were separated into two categories: 'unorganized' and 'organized' (2). Enzymes are divided into six categories. There are four types of hydrolysis enzymes, including amylase and protease. Amylase hydrolyzes starch and transforms dextrin to maltose, which is ultimately turned to glucose and other simple sugars (3). Kirchhoff was the first to introduce amylase3, a starch hydrolytic enzyme. Amylase, an extracellular enzyme, hydrolyzes the α-1,, 4 glyosidic link. Amylase can react on the inside of molecules, which is known as Endo amylase, and on the exterior of molecules, which is known as Exo amylase (4). Amylase is classified into three groups: α- amylase, β- amylase, and γ- amylase. Amylase accounts for nearly 30% of all enzymes on the international market, and it is widely distributed across aerobic and anaerobic species (5). Amylase extracted from a wide variety of sources, including plants, animals, and microorganisms such as bacteria and fungi, because microorganisms are easily modified using r-DNA technology. This technology is used to increase the production of α- amylase by making changes to the gene, such as inserting the desired genetic sequence of the organism. *Bacillus sterothermophilus* is a kind of bacteria (6).
B. subtilis, B. amyloliqefuciens, and other bacteria such *as Escherichia spp, Proteus spp*., *Rhizobium spp*., and *Actinomycetes spp*. Fungi like *A. Niger*, *Rhizopus*, *Mucor*, *Neurospora*, and *Penicillium* produce more amylase than yeast like *Candida.* Amylase is widely used in a variety of sectors such as paper, starch degradation, pharmaceutical, detergent industries, food industries starch processing, fermentation process, textile industries, and many others (7,8).
In this investigation, marine samples were collected, and alkaliphilic bacteria were isolated and alkaliphilic amylase was obtained. This amylase is active at high pH. This includes biochemical testing and purification, as well as optimization, production, and purification. This study may contribute in the isolation of industrially important enzyme with an advantage of it's survival in higher alkaline stress.

MATERIAL AND METHODS

Sample collection

The Arabian Sea water was collected from Kamboi [shree stamheshwar mhadev temple] latitude 23.674°N and longitude 72.0194°E, located in the south section of Gujrat, India.

Isolation and Screening of amylase producing bacteria

After collecting the sample, it was spread on the starch nutrient agar plate and incubated at 37°C for 48 hours. After incubation, the amylase producing bacteria were isolated based upon their ability of production of hydrolytic zone around the bacterial colony.

Morphological and Biochemical Characterization

Morphological characterization

The morphology of the bacterial isolate from the 24-hour old bacterial culture was studied using Gram's staining method.

Biochemical characterization

To investigate the isolate's characteristic features, the plate containing the isolated bacteria from the sea water sample was chosen and subjected to a series of biochemical tests, including the, Carbohydrate Catalase test, Citrate Utilization test Oxidase test, MR test, VP test, Indole test Fermentation for Sugars, Lactose, and Mannitol sugar, and the Triple Sugar Iron (TSI) test.

Optimization of bacterial growth

Effect of pH

To observe the effect of pH on the growth of bacteria, the isolate was inoculated in the medium having different pH in the range 6 to 12. The pH was adjusted using the different buffers such as Sodium acetate, Tris-HCL, Glycin-NaOH and Sodium carbonate then incubated for 24 hours at 37°C. The growth of bacteria was measured using UV-visible spectroscopy at the wavelength of 600 nm.

Effect of Temperature

To observe the effect of temperature on the growth of bacteria , the isolate was inoculated in the medium having pH 10 and incubated at different temperature such as 25°C, 37°C and 55°C for 24 hours. The bacterial growth was measured using UV-visible spectroscopy at the wavelength of 600 nm.

Effect of Substrate Concentration

To observe the effect of substrate concentration on the growth of bacteria, the isolate was inoculated in the medium having various concentration of soluble starch from 1% to 5%. The pH of medium was set at 10 and then it was incubated for 24 hours at 37° C.

Enzyme assay

The enzyme activity was determined using DNSA assay. In a 0.5 mL reaction mixture with 1% starch as the substrate, Sodium acetate buffer (50 mM) The buffer has pH 6.0., and 1% starch as the substrate, the enzyme was diluted appropriately. The released sugar was determined by adding 0.5 ml 3,5-dinitro salicylic acid (DNS) and boiling for 10 minutes to generate color after a 20-minute incubation at 37 °C (9). Under test conditions, 1 U of enzymatic activity is defined as the g of product formed per ml of culture in 1 minute. At 540 nm, the absorbance of the mixture was measured. The total protein concentration was identified using Bradford's method.

Partial Purification of Amylase

Crude enzyme extract

A 24-hour-old bacterial culture broth was centrifuged at 4 °C for 20 minutes at 10,000 rpm. to start the purification process. It was determined that this enzyme is a crude enzyme.

Ammonium sulphate precipitation

The crude extract was brought to a final concentration of 100% (w/v) with continual agitation at minimum temperature. After centrifugation for 20 minutes at 10000 rpm and 4 °C, the protein precipitate was recovered. The protein precipitate was dissolved in a little amount of Tris-HCl buffer 20 mM (pH 7.5) which was further subjected to dialysis in the same buffer.

RESUL AND DISCUTION

Isolation and Screening of amylase producing bacteria

A total of 62 colonies were obtained from the sea water sample out of which 10 colonies were able to produce the amylase enzyme. As shwon in Figure 1. the amylase producing bacteria was able to produce the halzo zone around the colony when grown on starch agar plate and subsequent treatment of iodine. The isolate which produced the biggest halo zone was further selected for studies.

Morphological Characterization of bacterial isolate

The morphological chracterization of isolate was carried out using Gram's staining. As shown in Figure 2, The isolate was Gram positive, rod-shaped, and has a single arrangement that can be seen under a microscope.

Biochemical test

To unfold the biochemical characteristics of bacteria various biochemical tests carried out. It was found that the isolate was MR, VP, oxidase and indole negative whereas, it provided positive results for catalase test, carbohydrate fermentation, TSI and citrate utilization. The bacteria showed positive result in motility test which reveals that isolate is motile (shown in Table 1).

Optimization of bacterial Growth

Effect of pH on bacterial growth

To observe the effect of pH on bacterial growth, the isolate was inoculated in the medium having the pH range from 5 to 12. As shown in Figure 5. The maximum growth of isolate was observed in the medium having pH 10. The bacterial growth was subsequently increasing with increased pH and at pH 10 it showed highest mass and later it started declining. Therefore, it shows that the pH 10 was optimum for the growth of isolate and it can grow well in the pH range of 6 to 11.

Effect of Temperature on bacterial growth:

To observe the temperature's effect on bacterial growth, the isolate was inoculated in the medium having the pH 10 at varied temperature from 25°C to 55°C. As shown in Figure 4, The maximum growth of isolate was observed when it was incubated at 37°C. The bacterial growth was subsequently increasing with increased temperature and at temp 37° C it showed optimum growth. Later it started declining. Therefore, it shows that the temp 37°C was optimum for the growth of isolate and it can grow well in the temp range of 25°C to 55°C.

Effect of substrate concentration on bacterial growth

To study that the concentration of substrate effects bacterial growth, the isolate was inoculated in the medium having various soluble starch concentration in the range of 1% to 5%. As shown in Figure 3. The maximum growth of isolate was observed when it was incubated in the 3% starch soluble medium. The bacterial growth was subsequently increasing with increased concentration of starch and at 3% it showed optimum growth. Later it started declining. Therefore, it shows that the 3% soluble starch was optimum for the growth of isolate and it can grow well in the starch concentration range of 1% to 5%.

Enzyme Assay

The enzyme activity assay was done by DNSA method. It was found that crude extract of enzyme was having the estimated activity of 161.5U/mL. The total protein concentration was observed to be as 6.30 mg/mL

PARTIAL PURIFICATION OF ENZYME

Ammonium Sulphate Precipitation and dialysis

Ammonium sulphate precipitation and dialysis were conducted on the crude enzyme sample. The ammonium sulphate precipitation increased the enzyme activity to 172.36 U/ml. The ammonium sulphate chelates the proteins and increases the total protein concentration as well. The similar result was observed and the total protein concentration was observed to be as 8.64 mg/ml.

Table 1. Biochemical test

Biochemical test	**result**
Indole production	-
Methyl Red test	-
Vogous – Proskauer test	-
Citrate Utilization test	+
Triple Sugar iron test	+
Carbohydrate fermentation (Lactose)	+
Carbohydrate fermentation (sucrose)	+
Carbohydrate fermentation (Mannitol)	-
Motility test	+
Catalase test	+
Oxidase test	-

Figure 1. Isolated amylase producing colony

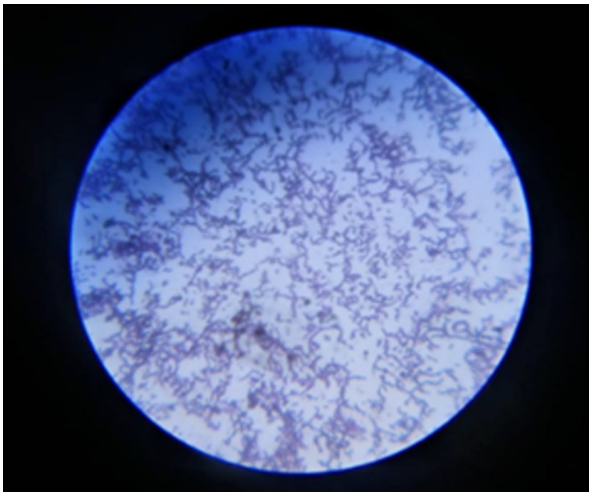

Figure 2. Gram staining of isolate

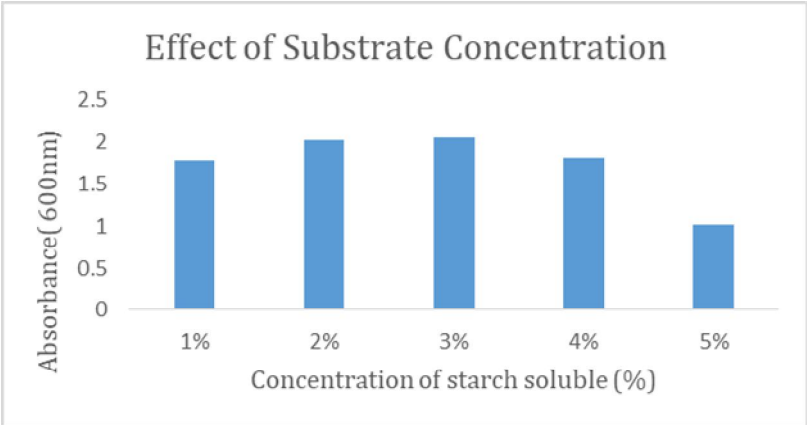

Figure 3. Effect of substrate concentration on bacterial growth

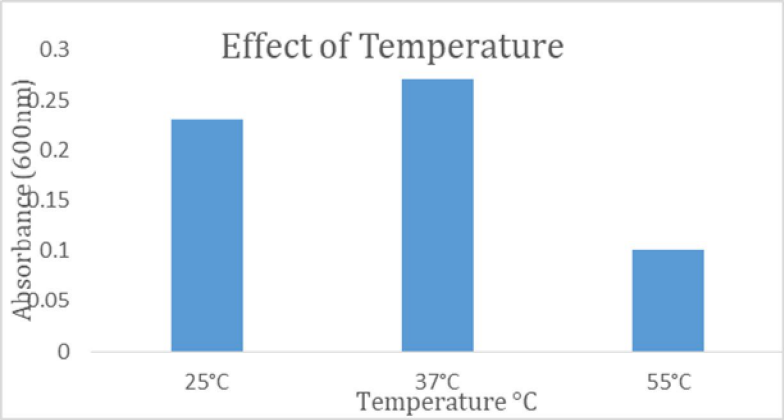

Figure 4. Effect of Temperature on bacterial growth

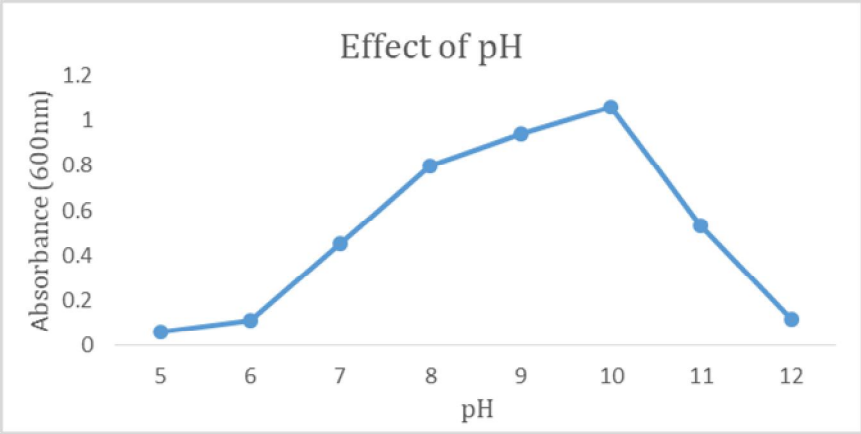

Figure 5. Effect of pH on bacterial growth

CONCLUSION

In comparison to neutrophilic amylase, alkaliphilic amylase is relatively stable. The alkaliphilic amylase produced from the bacterium isolated from kavi kambi has the crude enzyme activity of 161.5 U/ml. The growth optimization results revealed that the isolate possess optimum pH, temp and substrate concentration of 10, 37°C, and 3% respectively. The partial purification increased the enzyme activity upto 172.36 U/mL. Therefore, the amylase isolated from marine water sample may be used for the commercial production.

ACKNOWLEDGEMENT

The authors of the paper wish to express their gratitude to the administrators of Parul University in Waghodia, Vadodara- Gujarat, India for providing the required resources to carry out the research mentioned in this paper.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Kumari, A., Singh, K., & M Kayastha, A. (2012). α-Amylase: General properties, mechanism and biotechnological applications-A review. *Current Biotechnology*, *1*(1), 98-107.
2. Chavan, S., & Wadatkar, C. M. (2014). Isolation, Purification and Characterisation of Alpha amylase From Phaseolus Aconitifolius. *Journal of Advanced Drug Delivery*, *1*(3), 114-121..
3. Van Der Maarel, M. J., Van der Veen, B., Uitdehaag, J. C., Leemhuis, H., & Dijkhuizen, L. (2002). Properties and applications of starch-converting enzymes of the α-amylase family. *Journal of biotechnology*, *94*(2), 137-155.
4. Robyt, J. F., & John, F. (1998). *Essentials of carbohydrate chemistry*. Springer Science & Business Media.
5. 5.Thompson, D. B. (2000). On the non-random nature of amylopectin branching. *Carbohydrate Polymers*, *43*(3), 223-239.
6. Windish, W. W., & Mhatre, N. S. (1965). Microbial amylases. *Advances in applied microbiology*, *7*, 273-304.

7. Hashim, S. O., Delgado, O. D., Martínez, M. A., Kaul, R. H., Mulaa, F. J., & Mattiasson, B. (2005). Alkaline active maltohexaose-forming α-amylase from Bacillus halodurans LBK 34. *Enzyme and Microbial Technology*, *36*(1), 139-146.
8. Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T., Singh, D., & Mohan, R. (2000). Advances in microbial amylases. *Biotechnology and applied biochemistry*, *31*(2), 135-152.
9. Kadziola,s A., Søgaard, M., Svensson, B., & Haser, R. (1998). Molecular structure of a barley α-amylase-inhibitor complex: implications for starch binding and catalysis. *Journal of Molecular Biology*, *278*(1), 205-217.
10. Mishra, S., & Behera, N. (2008). Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. *African journal of Biotechnology*, *7*(18).
11. Saxena, R. K., Dutt, K., Agarwal, L., & Nayyar, P. (2007). A highly thermostable and alkaline amylase from a Bacillus sp. PN5. *Bioresource Technology*, *98*(2), 260-265.
12. Baltaci, M. O., Genc, B., Arslan, S., Adiguzel, G., & Adiguzel, A. (2017). Isolation and characterization of thermophilic bacteria from geothermal areas in Turkey and preliminary research on biotechnologically important enzyme production. *Geomicrobiology journal*, *34*(1), 53-62.
13. Singh, G., Bhalla, A., Kaur, P., Capalash, N., & Sharma, P. (2011). Laccase from prokaryotes: a new source for an old enzyme. *Reviews in Environmental Science and Bio/technology*, *10*(4), 309-326.

CITATION OF THIS ARTICLE

V Vala, P Andhare, I Bhattacharya, A Thakur, D Upadhyay. Isolation and Optimization of Alkaline Amylase from Marine Bacteria. Bull. Env.Pharmacol. Life Sci., Vol Spl Issue [3] 2022: 160-165