BIOCHEMICAL ANALYSIS AND OPTIMIZATION OF AEROMONAS AND BACILLUS SPECIES FOR ALPHA - AMYLASE PRODUCTION ISOLATED FROM SOIL SAMPLES OF RANCHI, JHARKHAND

Sapna Suman[1]*, Ladly Rani[2], Md Zakir Hussain[3], Shweta Kashyap[4], Rupa Verma[5]

Abstract

The optimization of extracellular α-amylase production by six different Aeromonas and Bacillus species from different soil samples was conducted. The bacterial strains Aeromonas *jandaei* (accession no. OR136166), Aeromonas *hydrophila* (Accession no. OR136168), Aeromonas *veronii* (accession no OR136284), Bacillus *subtilis* (accession no. OR357659), Bacillus *cereus* (accession no. OR136189) and Bacillus *amyloliquefaciens* (accession no. OR244384) underwent biochemical characterization. In this study we have found that among all the three species of Bacillus, Bacillus *subtilis* showed the maximum enzyme activity and for Aeromonas species it is Aeromonas *veronii* which displayed the maximum enzyme activity. Bacillus *subtilis* displayed its maximum α-amylase activity of 8.96 µmol/min at pH 7, and temperature at 35°C after 24 hours of incubation. And Aeromonas *veronii* displayed the maximum α-amylase activity of 7.58 µmol/min at pH 7, 35^0C temperature after 24 hours of incubation.

Key words: amylase, starch, enzyme activity, biochemical analysis

[1*,2,3,4,5]Department of Botany, Ranchi University, Ranchi, Jharkhand, India

***Corresponding Author:** Sapna Suman
Email: sapna.minz01@gmail.com.

DOI: - 10.53555/ecb/2022.11.11.133

Introduction

Starch hydrolysis primarily resulted in maltose, indicating the presence of α-amylase activity. The conversion of starch into dextrin and sugar is facilitated by enzymes derived from plants, animals, bacteria, and fungi. Microorganisms play a crucial role in the soil microflora due to their abundance and the diversity of their metabolic activities (Blaine et al 1992, Torsvil et al 1990). They play a crucial role in the biogeochemical cycles of both major and minor elements, thus actively participating in energy and nutrient exchanges within the soil (Ward et al 1995). While there are numerous microbial sources for amylase production, Bacillus strains stand out due to their ability to generate substantial quantities of amylase. They account for approximately 60% of commercially available enzymes. The starch industry primarily relies on amylases produced by Bacillus subtilis and Bacillus *mesentericus* for their thermostability and efficiency. Bacillus sp., sourced from soil, has been documented as a producer of α-amylase, demonstrating a high yield through starch hydrolysis and biochemical techniques (Aneela et al 2015). α-Amylase (1,4-α-D-glucan glucanohydrolase; EC3.2.1.1) is an endo-acting enzyme that catalyses the hydrolysis of starch by breaking α-1,4-glucosidic linkages (Tester et al 2004). Bacteria have emerged as a significant resource for generating thermostable α-amylase with superior characteristics compared to fungi (Prakash et al 2010). Choosing the appropriate organism is crucial for achieving a high yield of desired enzymes. The continuous development of new microbial strains for industrial enzyme production involves utilizing cost-effective carbon and nitrogen sources. Microorganisms are categorized into two main classes for amylases: α-amylase and glucoamylase (Hema et al 2006). α-amylase holds the top position among various extracellular enzymes, and its commercial applications have expanded across multiple sectors, including clinical, medicinal, and analytical chemistry. Starch-degrading bacteria play a crucial role in industries such as food, textiles, fermentation, and paper (Patel et al 2021). The isolation and manipulation of pure cultures of starch-degrading microorganisms from soil are highly significant in the field of biotechnology. Consequently, isolating and manipulating pure cultures from diverse waste materials holds manifold importance for various biotechnology industries. Major α-amylase-producing bacteria, such as Bacillus *subtilis*, B. *licheniformis*, B. *amyloliquefaciens*, B. *cereus*, and B. *megaterium*, as well as fungi like Aspergillus *Niger*, Penicillium, Rhizopus, Cephalosporium, and Neurospora, are pivotal in this context (Pandey et al 2003). Enzymes derived from microorganisms are regularly employed in numerous eco-friendly and economically viable industrial domains. Environmental pollution is no longer considered unavoidable in technological societies. The growing awareness of pollution's impacts and public pressure has prompted changes in both industry and government. There is a rising demand to substitute certain conventional chemical processes with biotechnological approaches utilizing microorganisms and enzymes like amylases, xylanases, cellulases, and mannanase. These alternatives not only offer an economically feasible option but are also more environmentally sustainable (Hoondal et al 2002). They have been utilized in various sectors such as food, baking, brewing, textiles, and paper industries. The growing use and demand for alpha-amylase in different industries have intensified the emphasis on boosting local enzyme production and exploring faster processes (Ramchandran et al 2004).

Material and methods

Production medium and extraction of crude enzyme

Fig 1. Pure culture of all the six bacterial isolates

A small portion of the bacterial culture was transferred from starch-nutrient agar slants to a starch-nutrient broth at a pH of 7 to activate it. This activation was carried out in a shaker at 40°C with a rotation speed of 120 rpm for 24 hours. The fermentation medium was composed of the following components per litre: soluble starch (10 g), peptone (5 g), (NH4)2 SO4 (2 g), KH2PO4 (1 g), K2HPO4 (2 g), and MgCl2 (0.01 g), all maintained at a pH of 7. The activated culture was used to inoculate the fermentation medium at a volume of 20%, and it was then incubated in a shaker at 37°C for 24 hours (Yandri et al 2010).

Upon concluding the fermentation period, the culture medium was subjected to centrifugation at 10,000 rpm for 15 minutes to yield a crude extract, which served as the source of the enzyme

Biochemical analysis of screened bacteria

The amylase-producing bacteria, which underwent screening, were subjected to morphological characterization using the Gram's staining method. Additionally, they were biochemically characterized through various tests including the catalase test, oxidase test (using the filter paper method), methyl red test, Voges-Proskauer (VP) test, and urease test (Holt et al 1994).

Enzyme assay:

The α-amylase activity was determined using the dinitrosalicylic acid (DNS) method, following the procedure outlined by Fisher and Stein (Chi et al, 2009). The un-inoculated (UN) culture broth served as the blank. Amylase activity was defined as the amount of enzyme needed to catalyze the release of reducing sugar equivalent to one mole of maltose per minute under the specified assay conditions. The assay involved incubating 1 ml of the enzyme sample, 1 ml of substrate, and 0.5 ml of dinitrosalicylic acid on a water bath for 15 minutes. Subsequently, the mixture was cooled to room temperature, and the activity was measured at an absorbance of 540 nm. The measurement of amylase enzyme activity was conducted using a method that combines elements from the procedures outlined by Naguib and Qureshi (Naguib et al 1964, Qureshi et al 2013).

Determination of the Optimal Conditions for α-Amylase Production:

Optimal Temperature: The impact of temperature on α-amylase activity was investigated by varying the incubation temperature within the range of 25ºC to 45°C in increments of 5 degrees.

Optimal pH: The synthesis of α-amylase was influenced by various pH ranges, including 5 to 9 using acetate buffer, pH 6.0 to 7.5 using phosphate buffer, and Tris-HCl for pH 8.0 to 8.5(Alariya et al., 2013).

Result and discussion

Biochemical analysis of bacteria

Sr. No.	Bacterial isolates	Gram staining
1.	Aeromonas *hydrophila* (Accession no. OR136168)	Gram negative
2.	Aeromonas *jandaei* (accession no. OR136166)	Gram negative
3.	Aeromonas *veronii* (accession no OR136284)	Gram negative
4.	Bacillus *subtilis* (accession no. OR357659)	Gram positive
5.	Bacillus *cereus* (accession no. OR136189)	Gram positive
6.	Bacillus *amyloliquefaciens* (accession no. OR244384)	Gram positive

Bacterial isolates	Catalase test	Oxidase test	Methyl Red test	Voges Proskauer (VP)test	Urease test
Aeromonas *jandaei*	positive	positive	positive	positive	positive
Aeromonas *hydrophila*	positive	positive	positive	positive	negative
Aeromonas *veronii*	positive	positive	positive	positive	positive
Bacillus *subtilis*	positive	positive	negative	positive	negative
Bacillus *cereus*	positive	positive	negative	positive	negative
Bacillus *amyloliquefaciens*	positive	positive	negative	positive	negative

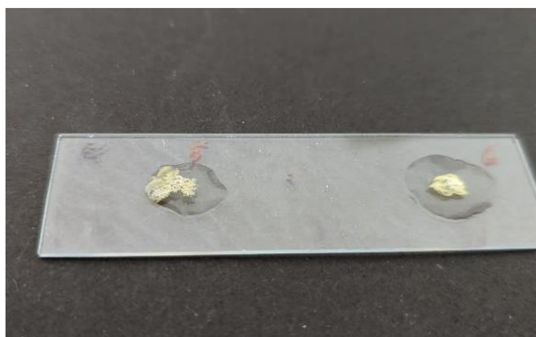


Fig 2: catalase test

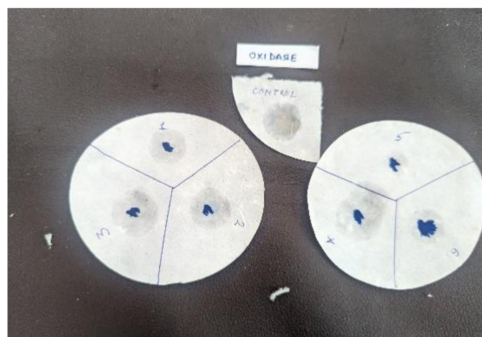


Fig 3: Oxidase test

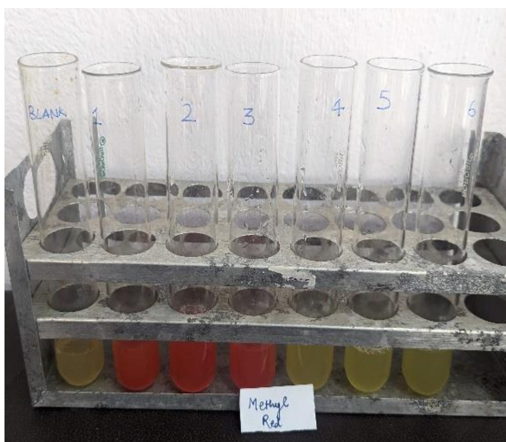


Fig 4: MR test

Fig 5: VP test

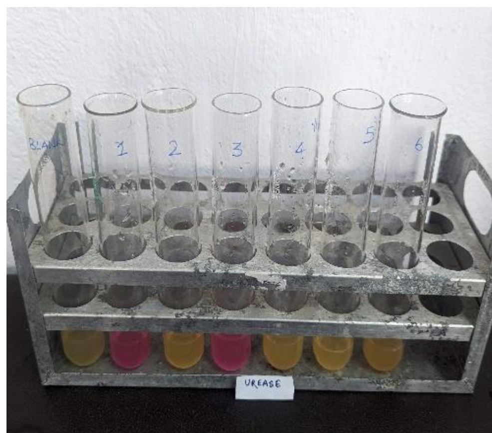
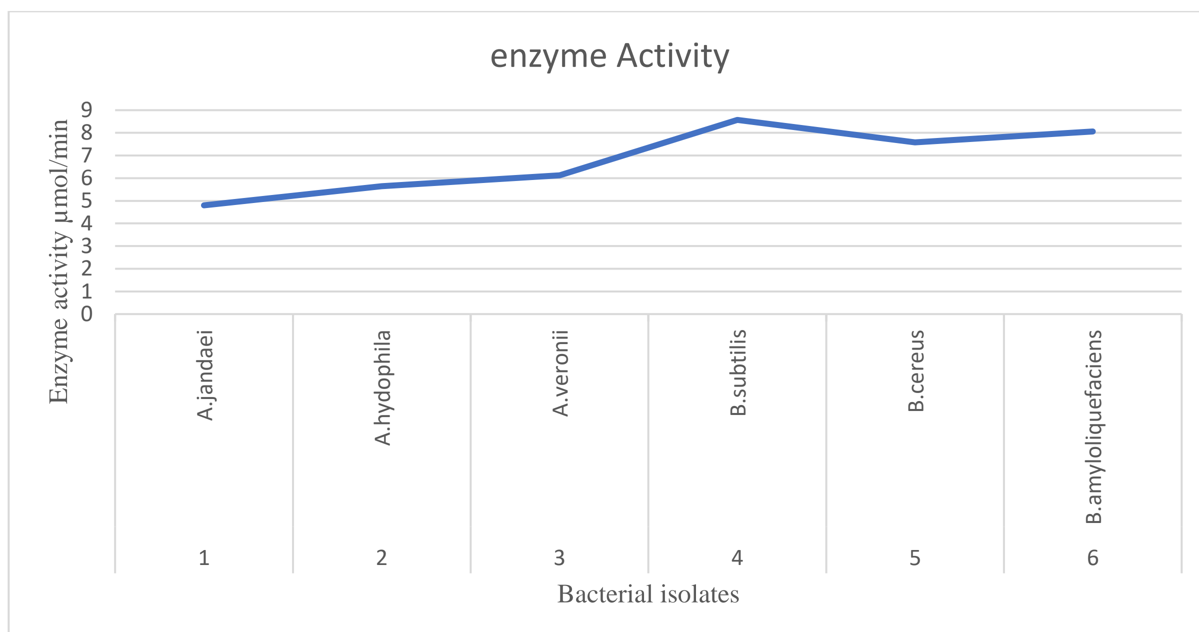


Fig 6: Urease test

Enzyme activity

Table 1: Enzyme activity (µmol/min) of all the six bacterial isolates

Sr. no	Bacterial isolates	Enzyme activity (µmol/min)
1	Aeromonas *jandaei*	4.8
2	Aeromonas *hydrophila*	5.64
3	Aeromonas *veronii*	6.12
4	Bacillus *subtilis*	8.57
5	Bacillus *cereus*	7.58
6	Bacillus *amyloliquefaciens*	8.06

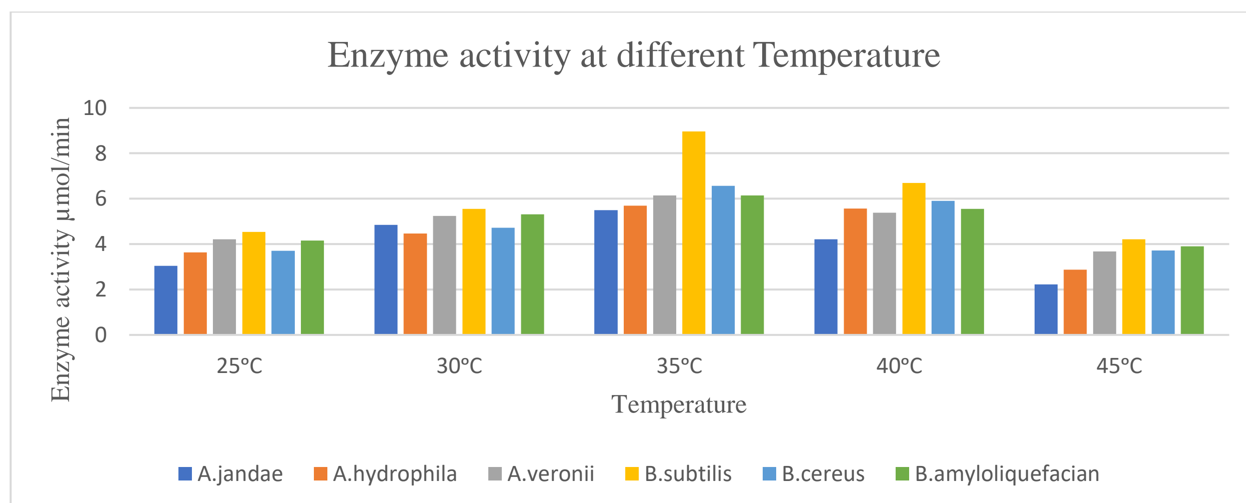


Graph 1: Enzyme activity of all the six bacterial isolates.

Optimal Conditions for Amylase Production

Table 2: Enzyme activity (µmol/min) at different temperature

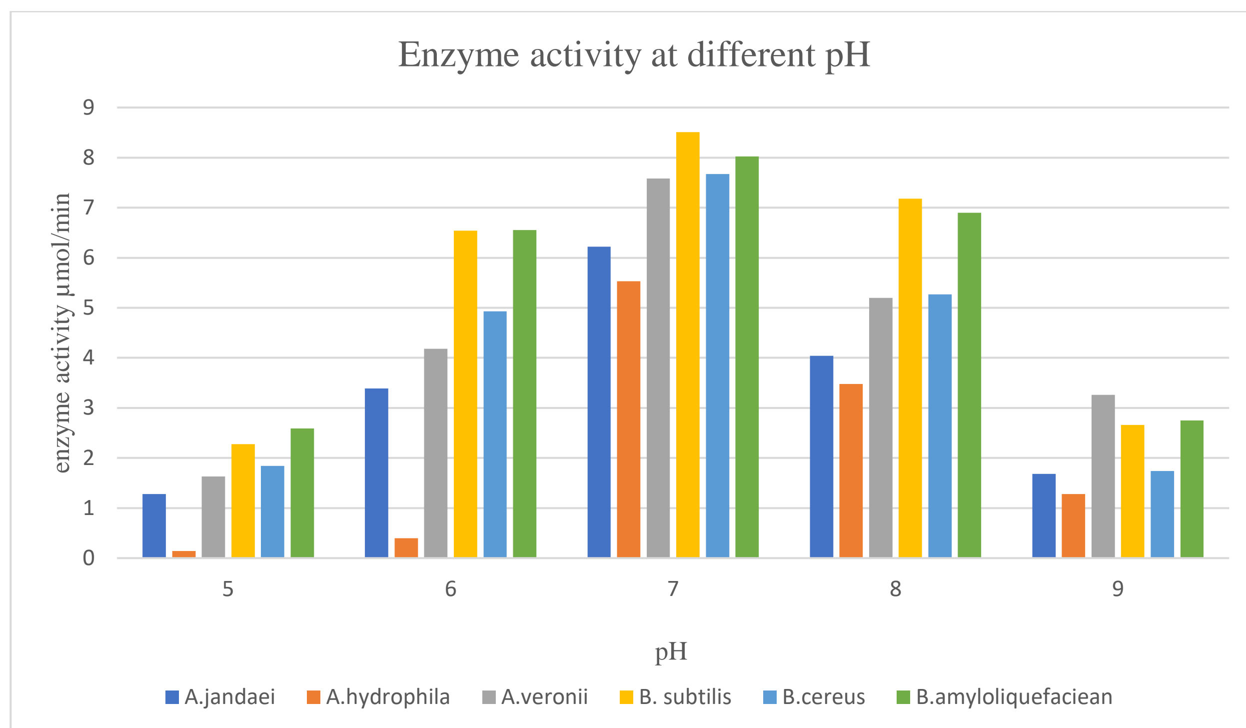
Temperature	Aeromonas *jandaei*	Aeromonas *hydrophila*	Aeromonas *veronii*	Bacillus *subtilis*	Bacillus *cereus*	Bacillus *amyloliquefaciens*
25ºC	3.04	3.64	4.22	4.54	3.70	4.16
30ºC	4.85	4.46	5.24	5.55	4.72	5.31
35ºC	5.49	5.69	6.15	8.96	6.57	6.14
40ºC	4.22	5.57	5.38	6.70	5.90	5.55
45ºC	2.22	2.87	3.68	4.22	3.72	3.91



Graph 2: Enzyme activity at different temperature

Table 3: Enzyme activity (µmol/min) at different pH

pH	Aeromonas *jandaei*	Aeromonas *hydrophila*	Aeromonas *veronii*	Bacillus *subtilis*	Bacillus *cereus*	Bacillus *amyloliquefaciens*
5	1.28	0.14	1.63	2.28	1.84	2.59
6	3.39	0.40	4.18	6.54	4.93	6.55
7	6.22	5.53	7.58	8.51	7.67	8.02
8	4.04	3.48	5.20	7.18	5.27	6.90
9	1.68	1.28	3.26	2.66	1.74	2.75



Graph 3: Enzyme activity at different pH

Conclusion

In this study all the Aeromonas species were found to be gram's negative and all the bacillus species are gram's positive. Aeromona species are oxidase, catalase, MR, VP and urease positive, except for Aeromona *jandaei* which is urease negative. And for Bacillus species all the species are oxidase, catalase and VP positive and MR and urease negative. Upon optimization, among all three Bacillus species, Bacillus *subtilis,* displayed the maximum amylase activity which was 8.96 µmol/min at pH 7.0, demonstrating considerable stability in alkaline conditions and temperature at 35°C after 24 hours of incubation. And among Aeromonas species Aeromonas *veronii* showed the maximum amylase activity of 7.58 µmol/min at pH 7, 35^{0}C temperature after 24 hours of incubation. From this study we can conclude that soil of Ranchi, Jharkhand have the bacteria which are highly capable of producing α-amylase which can be used for various commercial purposes.

Acknowledgment

I Sapna Suman want to acknowledge the HOD of the University Department of Botany Dr. Kunul Kandir for providing me with the lab facility. I want to thank Dr. Ladly Rani for her guidance and support. Lastly, I want to thank my lab mates Md. Zakir Hussain and Shweta Kashyap.

Reference

- A. Hema, T. Ujjval, and P. Kamlesh, "Alpha amylase production by *Bacillus cereus* MTCC 1305 using solid-state fermentation," *Food Technology and Biotechnology*, vol. 44, no. 2, pp. 241–245, 2006.
- A. Pandey, "Solid-state fermentation," *Biochemical Engineering Journal*, vol. 13, no. 2-3, pp. 81–84, 2003.
- Aneela Rehman, Asma Saeed. Isolation and screening of amylase producing Bacillus species from soil. International Journal of Advanced Research. 2015; 3(4): 151-164.
- Blaine Metting Jr F. (Ed.). Soil Microbial Ecology. Marcel Dekker, Inc, New York, 1992, pp. 3–95.
- Hoondal *,2002*, Study on interaction of -amylase from *Bacillus subtilis* with cetyl.
- J. G. Holt, *Bergey's Manual of Determinative Bacteriology*, Lippincott Williams and Wilkins, Baltimore, MA, USA, 9th edition, 1994.
- Naguib MI 1964 Effect of sevin on the carbohydrate and nitrogen metabolism during the germination of cotton seeds *Indian J. Exp. Biol* **2**:149–152.
- Patel D, Upadhayay D, Marchawala F, Bhattacharya I and Andhare P," review on isolation, identification of amylase produced from soil bacteria", International journal of Biology, Pharmacy and Allied sciences(IJBPAS), April, Special Issue, 2021, 10(4): 29-41
- Prakash O and Jaiswal N 2010 *Appl. Biochem. Biotechnol.* **160** 2401.

- Qureshi AS, Bhutto M A, Khushk I, and Dahot M U 2013 Optimization of cultural conditions for protease production by *Bacillus subtilis* EFRL 01 *Afr. J. Biotechnol* 10**:**5173–5181.
- Ramachandran, S., Patel, A.K., Nampoothiri, K.M., Francis, F., Nagy, V., Szakacs, G., Pandey, A., (2004). Coconut oil cake – A potential raw material for the production of α-amylase, Bioresour. Technol., 93, 169–174.
- Tester R. F., Karkalas J. and Qi X. (2004). Starch–composition, fine structure and architecture. Journal of cereal science, 39: 151-165.
- Torsvik V., Goskoyr J., Daae F.L., High diversity in DNA of soil bacteria, Appl. Environ. Microbiol.56 (1990) 782–787(12)
- Ward-Raynet N., Rainey F.A et al.,1995.
- Yandri, Suhartati T and Hadi S. 2010 *Eur. J. Sci. Res.* **39** (1) 64
- Z. Chi, Z. Chi, G. Liu, F. Wang, L. Ju, and T. Zhang, “Saccharomycopsis fibuligera and its applications in biotechnology,” *Biotechnology Advances*, vol. 27, no. 4, pp. 423–431, 2009.