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Optimization of the physicochemical parameters for production of pectinase by *Aspergillus niger AG-1*

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Abstract

Chemical processes make it very difficult to generate many complicated chemical compounds, however biosynthetic pathways of microorganisms can be used to make these complex chemicals. This study aims to isolate fungi from rhizospheric soil habitat for commercial application in the industrial sector. Enzymes known as pectinases have numerous industrial and commercial uses. Fungi-produced pectinases convert the pectin found in plant material into simple chemical compounds. The fungal cultures were isolated from rhizospheric soil. Among all fungal isolates one isolate was exhibiting highest pectinase activity in screening which was primarily designates as AG-1 and further identified as *Aspergillus niger AG-1* by molecular identification. The optimisation of many physico-chemical parameters enhanced ability of *Aspergillus niger AG-1* to produce pectinases. At 35^{0}C, pectinase activity peaked between 56 and 64 hours later. The isolated fungus produced more increased pectinase when the pH was at pH 6.0, along with 3% inoculum volume and 2% salt concentration. Maltose and yeast extract, respectively, were the sources of carbon and nitrogen that resulted in increased enzyme activity. Due to its great productivity, pectinases produced by *Aspergillus niger AG-1* are ideal for manufacturing on an industrial scale.

Keywords: Pectinases, *Aspergillus niger AG-1*, physico-chemical parameters, optimization, fermentation

Introduction

Microorganisms are capable of producing many value-added products through the biosynthetic pathways (Du et al., 2011). These products have many applications in the field of pharmaceuticals (Banat et al., 2000), food and beverage industry (KV, 2022), agriculture (Demain, 1983) and many more. These products may include amino acids (Hermann, 2003), organic acids (Wendisch et al., 2006), vitamins (Burkholder et al., 1942), drugs (Wang et al., 2021) etc. Use of living entities instead of chemical methods for the production of such compounds has many advantages like avoidance of chemicals and chemical processes, ease of operation and handling, substrate specificity and hence lower proportion of by-product formation (Brahmachari et al., 2016). Many complex chemical compounds are very difficult to produce through chemical processes but biosynthetic pathways of microorganisms can be exploited to produce such complex compounds. The yield can also be increased through biosynthetic processes with the application of many bio-engineering techniques available now (Kataria et al., 2021). It is estimated that about 1% of bacteria and 5% of fungi have been found to be cultivated in laboratory (Bull et al., 1992). The optimization of the growth parameters is essential at such instances to get the higher yields (Fernandes et al., 2020).

There are many important enzymes like amylase (Van Der Maarel et al., 2002), protease (Singh et al., 2016), lipase (Ismailet al., 2020), xylanase (Polizeli et al., 2005), cellulase (Sharma et al., 2016), chitinase (Singh et al., 2021), pectinase etc. used commercially in industries. Pectinases have applications in fruit and vegetable processing industries. Pectinases enzymes were primarily used at homes and the first industrial application of pectinase for wine and fruit juice preparation on commercial scale was reported in 1930 (Kashyap et al., 2001). Pectinase is used for clarification of

fruit juices and obtaining stable fruit juice which ultimately increases the yield. In 1995, pectinase contributed 75% of the total estimated sale value of the industrially useful enzymes and therefore on commercial scale, it can be considered as one of the most useful enzyme (Kashyap et al., 2001). Other industrial applications of pectinases include animal feed, coating, pharmaceuticals, retting and degumming of fiber crops, treatment of pectic wastewater, paper making, production of Japanese paper, oil extraction, coffee and tea fermentation (Hoondal et al., 2002).

1.1 Pectin and pectinolytic enzymes:

Cell wall of the plants is composed mainly of polysaccharides and proteins. Polysaccharides include cellulose, hemicellulose and pectin. Pectin is major cell wall component in plants. It is also most complex macromolecule. Its complexity is due to presence of 17 different monosaccharaides and more than 20 different linkages among these (Ridley et al., 2001). Pectin acts as a cementing material for cell walls in the plants (Perez et al., 2003). Primary cell walls contain up to 35% pectin in amorphous form, 30% cellulose and 30% hemicellulose and 5% proteins. The structure of pectin changes during processing, storage or isolation from the plant material and therefore is very difficult to study it. Galacturonans and rhamnogalacturonans are the major components of pectic substances and carbon of galactate is oxidized to form a carboxyl group.

1.2 Microbial sources of pectinolytic enzymes

Pectinases are produced in many higher plants and also in plant parasites such as nematodes (Sakai et al., 1993). Pectinases produced by microorganisms play important roles in the process of phytopathology, symbiosis between plant and microbes and they are also useful in the decomposition of the dead plant materials. They contribute to the natural carbon cycle. Microorganisms produce multiple forms of pectinases differing in molecular mass and kinetic properties (Devi and Rao, 1996). This ability of microorganisms assists them to get adapted to the environmental conditions (Naessens & Vandamme, 2003). Pectinolytic enzymes are reported to be produced by many fungi (Aguilar & Huitron 1990), bacteria (Karbassi & Vaughn 1980) yeasts (Gainvors & Belarbi 1993). Most of the commercial production of pectinases is from fungi (Singh et al., 2021) and *Aspergillus niger* is the predominant species of fungi in industrial production of pectinases (Gummadi et al., 2003). Many fungi, bacteria and yeasts produce endo-polygalacturonases (Luh et al., 1951). Saprophytic fungi produce pectinases in abundance (Gummadi and Panda 2003). Endo-polygalacturonases have been reported to be produced by many microorganisms like *Rhizoctonia solani* Kuhn (Marcus et al., 1986), *Aureobasidium pullulans* (Sakai et al., 1984), *Fusarium moniliforme* (De Lorenzo et al., 1987), *Rhizopus stolonifer* (Manachini et al., 1987), *Neurospora crassa* (Polizeli et al., 1991), *Thermomyces lanuginosus* (Kumar and Palanivelu, 1999*), Aspergillus sp.* (Nagai et al., 2000), *Peacilomyces clavisporus* (Souza et al., 2003). Exo-polygalacturonases are found to be produced by a limited microbial species. They are reported to be produced by *Agrobacterium tumefaciens* (Rodrigues-Palenzuela et al., 1991), *Alternaria mali* (Nozaki et al., 1997), *Erwinia carotovora* (Paloma¨ki & Saarilahti, 1997), *Fusarium oxysporum* (Maceira et al., 1997), *Bacteroides thetaiotamicron* (Tierny et al., 1994), *Ralstonia solanacearum* (Huang and Allen, 1997), *Bacillus* sp. (Koboyashi et al., 2001).

The length of the incubation period, the temperature, the pH, and some dietary components like carbon and nitrogen all have an impact on the development of microbial enzymes. Scientists and researchers are particularly interested in the composition and amounts of Carbon and Nitrogen sources for the development of low-cost media. Designing a growth medium efficiently lowers production costs. This demonstrates how crucial growth media optimisation is to the efficient production of enzymes. Physical factors like temperature, pH, and incubation period are crucial in the synthesis of pectinase. In addition to these, dietary factors support pectinase synthesis. Pectinase makes approximately to 10% of all enzyme synthesis worldwide. Since citrus peel contains 24% pectin, it has been utilised as a raw material for the synthesis of pectinase for a very long time. The purpose of this study is to determine the ideal temperature, pH, incubation duration, and nutritional components (substrate, carbon, and nitrogen sources) for *Aspergillus niger* AG-1 to produce pectinase.

2. Materials and Methods

2.1. Screening and Isolation of pectinase producing microorganisms using unexploited agricultural wastes as substrate.

2.1.1 Collection of materials

The rhizospheric soil samples were collected aseptically from local cultivation lands during post-harvesting period at Pune District, Maharashtra. Soil sample from fruit waste dumping site were also collected. Soil samples were collected with the help of sterile spatula in sterile polythene bags and brought to the laboratory for further study. 1 g of the soil sample was mixed into 100 ml normal saline. Serial dilution of the sample from 10^{-1} to 10^{-6} were done and kept at 4^0C.

2.1.2 Isolation and Screening of pectinase producing fungi

A volume of 100 ul of each dilution was plated on to pectin agar medium plates and incubated at 37^0C for 24 hours (Sharma et al., 2011). Isolated colonies were selected on the basis of morpholoical differences and again the serial dilution up to 10^{-6} was done and inoculation was carried out on pectin agar medium plates. The pectinolytic activity of each isolate was detected on the basis of zone of clearance around the colony by flooding with iodine-potassium iodide solution (1.0 g iodine, 5.0 g potassium iodide, and 330 mL H_2O). Plates were washed with distilled water after 5 minutes incubation with intermittent shaking (Priya and Sashi 2014). Composition of Pectin Agar medium (in g/L) is as $NaNO_3$:1.0, KCl: 1.0, K_2HPO_4: 1.0, $MgSO_4$: 0.5, pectin: 10, yeat extract: 0.5, agar: 20, pH: 7.0 (Mukesh et al., 2012).

Isolates showing the maximum diameter of zone of clearance were selected for further study.

2.1.3 Characterization and identification of the isolated fungi

The microorganism showing pectinolytic activity were screened on the basis of higher cz (clear zone)/cs (colony size) ratio. For fungi, microscopic and macroscopic observations were done. Staining was done with lacto-phenol blue solution. Molecular analysis of the isolate was made on the basis of 18S rDNA sequences (Weisburg et al., 1991).

2.2 Preparation of fermentation medium

10 g of pretreated pectin-rich peels powder (PP) was moistened with 10 ml of mineral salt medium in 250 ml conicl flask. Inoculation was done with 1ml of inoculum suspension and incubated at 30^0C for 3 days. This inoculum suspension was prepared by adding 10 ml of 0.9% saline water in 4-day-old fungal culture and mixing thoroughly. 100 ml of 0.1 M sodium acetate buffer (pH 6) was added in fermented medium after a fixed incubation period and placed in shaking incubator for one hour. The contents of flask were filtered and filtrate was used for the assessment of pectinase.

2.2.1 Preparation of crude enzyme extract

15 ml aliquot of the filtrate (cell-free crude-pectinase extract) was taken out and centrifuged at 5000 rpm for 15 minutes. The cell-free crude-pectinase was stored at 4^0C and used for further enzyme assay.

2.2.2 Determination of pectinase activity: DNS method (Miller, 1959)

This method (Patil, N. P., & Chaudhari, 2010) involves the estimation of pectinase activity by release of reducing groups from pectin source.

Composition of DNS reagent: 1 g nitrosalicylic acid, 200 mg crystalline phenol and 50 mg sodium sulphate. Dissolve all these in 100 ml of NaOH (1%).

Other reagents required: Pure pectin (1%), Sodium acetate buffer with pH 4.8.

Procedure: Add 2 ml pure pectin (1%) with 0.5 ml of cell-free crude-pectinase extract in a test tube. Heat in water bath at 45^0C for 30 min. After cooling, add 2.5 ml of DNS reagent to it and heat for 5 min. Cool the reaction mixture and measure the absorbance with spectrophotometer at 540 nm. Plot a standard graph using polygalacturonic acid (PGA) (range 50 g/ml). Here, pectinase activity can be expressed as mg of PGA released per min per mg of protein.

2.3 Optimization of culture conditions for enzyme production (One-Variable-At-a-Time)

The effect of physico-chemical parameters like initial pH, incubation temperature, incubation time, inoculum volume, salinity, and extra-carbon and nitrogen sources on the yield of enzyme was studied and optimal conditions were assessed. All the experiments were carried out in triplicates.

2.3.1 Effect of initial pH on enzyme production

The initial pH of pectinase production medium was adjusted to 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8 with 1N HCl or 1N NaOH and 1% inoculum was used to inoculate pectinase production medium, incubated at 30^0C

(Holliman, 2012). Enzyme activity was assessed after 24, 48 and 72 hours.

2.3.2 Effect of incubation temperature on enzyme production

1% inoculum was used to inoculate pectinase production medium and incubated at temperature ranges of 25⁰C, 30⁰C, 37⁰C, 40⁰C, 45⁰C and 50⁰C with continuous shaking. Enzyme activity was assessed after 24, 48 and 72 hours (Torimiro and Okonji, 2013).

2.3.3 Effect of incubation time on enzyme production

1% inoculum was used to inoculate pectinase production medium and incubated at temperature 30⁰C for 24, 48 and 72 hours and enzyme activity was assessed.

2.3.4 Effect of inoculum volume on enzyme production

1%, 2%, 3%, 4% and 5% inoculum were used to inoculate pectinase production medium separately and incubated at temperature 30⁰C for 24, 48 and 72 hours and enzyme activity was assessed (Ahmed A. et al., 2019).

2.3.5 Effect of salinity on enzyme production

1% to 10% NaCl was added to the pectinase production medium. 1% inoculum was used for inoculation and incubated at temperature 30⁰C for 24, 48 and 72 and enzyme activity was assessed (Wang et al, 2019).

2.3.6 Effect of various extra-carbon and nitrogen sources on enzyme production

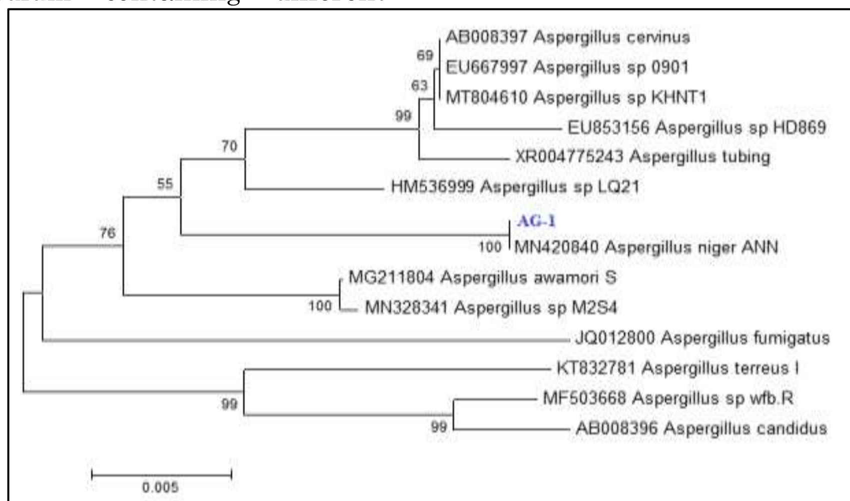
1% inoculum was inoculated to pectinase production medium containing different concentrations of extra-carbon sources (fructose, lactose, glucose, sucrose, xylose, dextrose, maltose) and nitrogen sources (urea, beef extract, peptone, yeast extract, ammonium sulphate, potassium nitrate, ammonium nitrate) at 0.5% final concentrations (Kalaichelvan 2012). Enzyme activity was assessed after 24, 48 and 72 hours (Wang et al, 2019).

3. Results and Discussion

The current study focuses on how different physical and chemical factors affect the synthesis of enzymes. Pectinase synthesis is controlled by physical, chemical, and nutritional variables. The best source of enzymes for human requirements is microbes. The concentration of the substrate, incubation period, temperature, pH, NaCl concentration, carbon and nitrogen sources and pH all have a significant impact on the microbial synthesis of enzymes.

3.1 Isolation and identification of the isolated fungi

Rhizospheric soil and soil sample from fruit waste dumping site was used to isolate the fungi, which were then tested for pectinase activity. The fungal isolate showing maximum activity of the pectinase enzyme was selected and studied for the morphological characteristics and 18S rDNA analysis. The strain was identified and designated as *Aspergillus niger AG-1* (Figure 1).



>***Aspergillus niger AG-1***

Figure 1: Phylogenetic placement of AG-1: Gene sequences showing relationships among strain AG-1 and the closer type strain species of *Aspergillus niger*. Numbers at nodes indicate percentage of bootstrap support based on a Neighbor-joining analysis of 1,000 resampled datasets. Bar 0.005 substitutions per nucleotide position. On the basis of 18S rDNA identification the isolate

AG-1 was identified as *Aspergillus niger AG-1*.

3.2 Effect of Incubation Time

Maximum pectinase activity (2.54 IU/ml) was seen after 56 hours of incubation in this experiment, where enzyme activity was steadily increased. Up until 64 hours, the enzyme activity was nearly steady; after that, it was slightly declined (Figure 2).

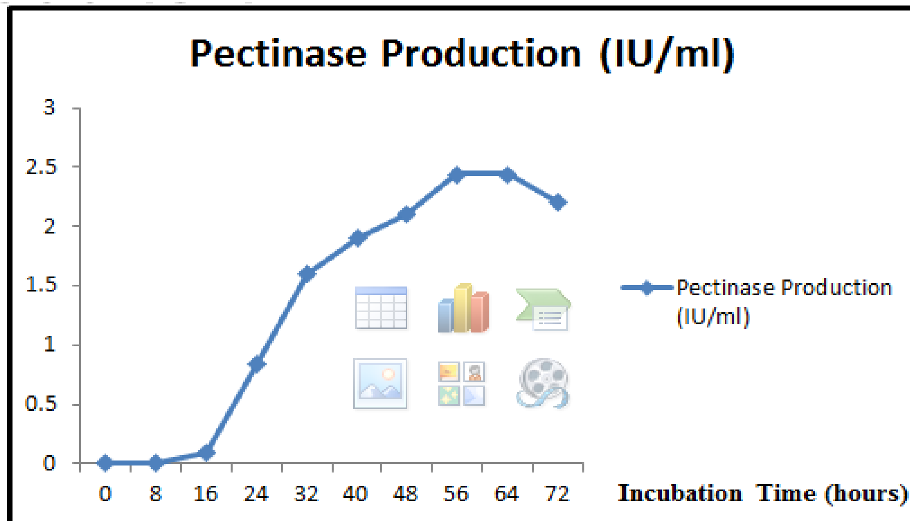


Figure 2: Effect of incubation time on pectinase production

3.3 Effect of Temperature

The studies were carried out between 25⁰C and 50⁰C temperatures. The optimal temperature for *Aspergillus niger AG-1* to thrive and produce pectinase was 35⁰C. The enzyme synthesis increased with temperature and was 1.12 IU/ml at room temperature. At 35⁰C, the maximum enzyme production of 2.52 IU/ml was noted. Any temperature increase above 35⁰C inhibits the microorganism's metabolic processes, resulting in less growth and enzyme synthesis. The findings of the current analysis are in close accord with the earlier reported statistics. Growth and the synthesis of enzymes were decreased at 45⁰C (Figure 3).

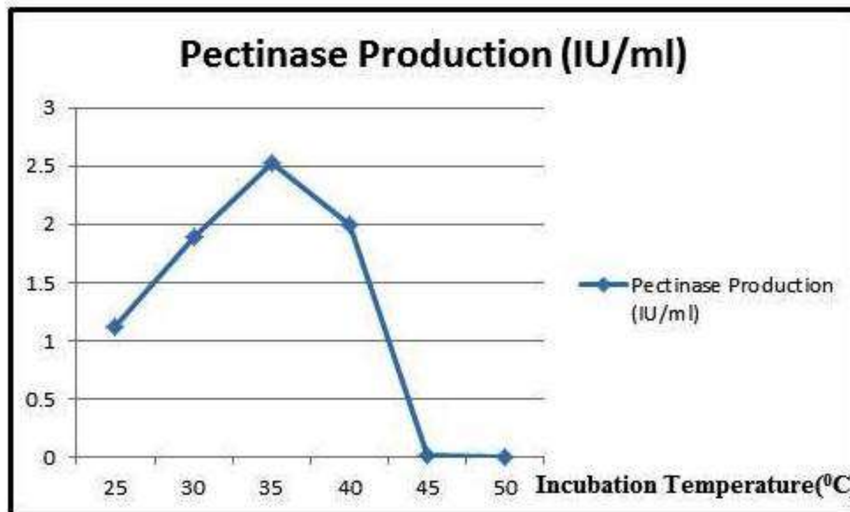


Figure 3: Effect of temperature on pectinase production

3.4 Effect of initial pH on enzyme production

The synthesis of pectinase by *Aspergillus niger AG-1* was carried out under various pH conditions ranging from pH 4 to 8, in order to determine the optimum pH. Beginning at pH 4, *Aspergillus niger AG-1* showed enzyme activity which gradually increased. At pH 6, the highest pectinase production (2.55 IU/ml) was observed which became stable at pH 6.5, After pH 7, enzyme synthesis started to decline, and pH 8 barely registered any pectinase synthesis (Figure 4).

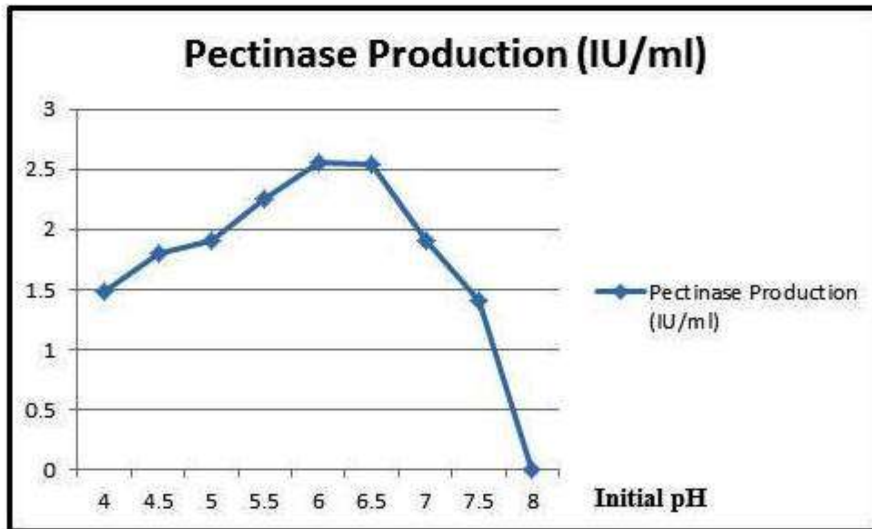


Figure 4: Effect of initial pH on pectinase production

3.5 Effect of salinity on enzyme production

The impact of salinity on enzyme synthesis and growth was investigated. NaCl concentrations ranging from 1% to 10.0% were investigated at various concentrations. With an increase in NaCl concentration from 1% to 2.0%, a gradual increase in enzyme synthesis was seen. As a result, the concentration of NaCl had an impact on the growth of the fungus and enzyme production. Finally, it was discovered that a 2% NaCl concentration was optimal for promoting development and the synthesis of pectinase enzyme which exhibited 2.52 IU/ml enzyme activity (Figure 5).

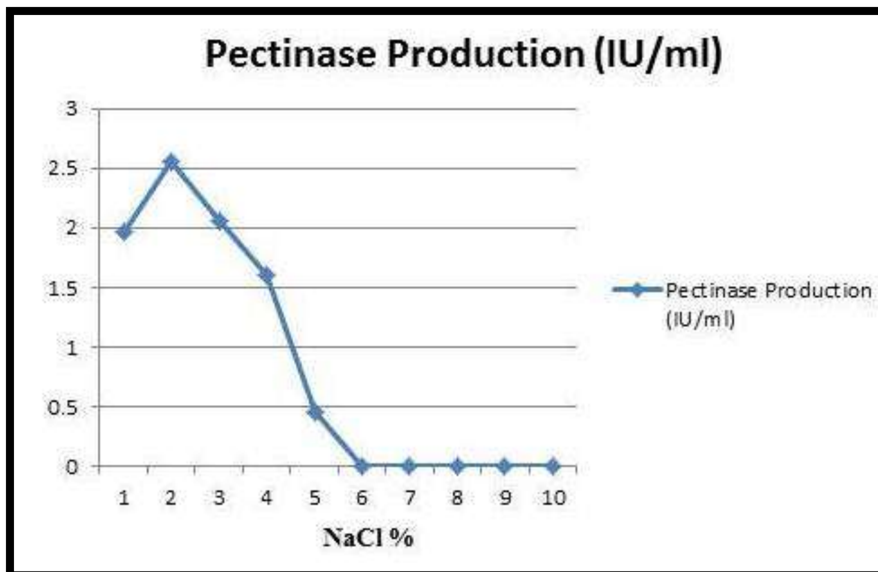


Figure 5: Effect of salinity on pectinase production

3.6 Effect of inoculum volume on enzyme production

The impact of inoculum volume on enzyme synthesis and growth was investigated. inoculum volume ranging from 1% to 5.0% were investigated for the synthesis of pectinase. With an increase in inoculum volume from 1% to 3.0%, a gradual increase in enzyme synthesis was seen. As a result, the inoculum volume had an impact on the enzyme production. Finally, it was discovered that a 3% inoculum volume was optimal for promoting development and the synthesis of pectinase enzyme (Figure 6).

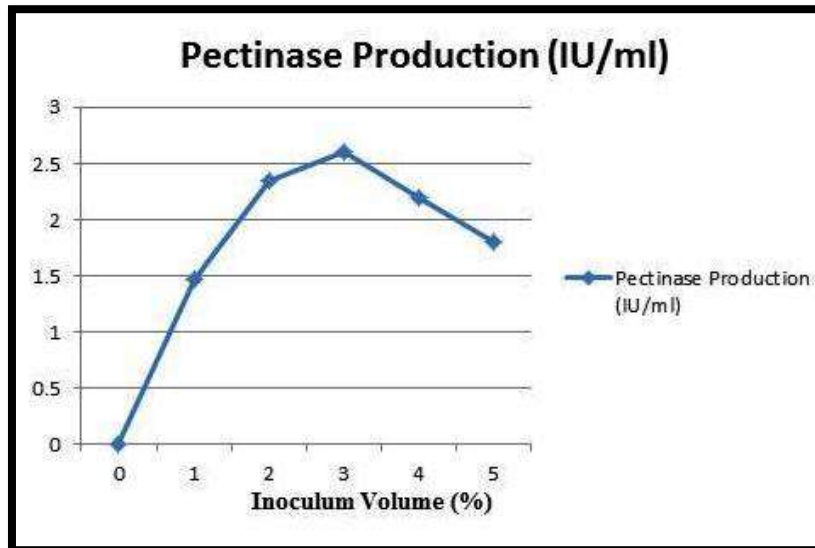


Figure 6: Effect of inoculum volume on pectinase production

3.7 Effect of various additional carbon and nitrogen sources on enzyme production

It was observed that when 0.5% of maltose was added to the pectinase production medium as an extra-carbon source, the pectinase yield improved (1.64 IU/ml), but no considerable effects were observed in case of other carbon sources such as fructose, lactose, sucrose, xylose or dextrose. It can be to the inhibitory effect of high concentrations of carbon (Naidu and Panda, 1998) (Figure 7 and 8).

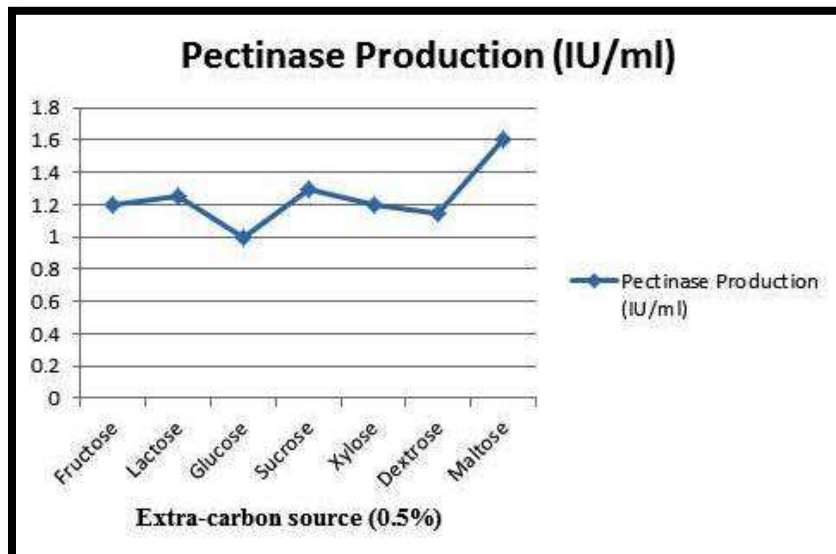


Figure 7: Effect of additional carbon source pectinase production

In order for fungi to grow and produce enzymes, the source of nitrogen in the growth media is extremely important. According to the current study, yeast extract is the best source of nitrogen for production of pectinase. The inclusion of yeast extract as an additional nitrogen source to the pectin agar medium resulted in the highest enzyme production (2.60 IU/ml).

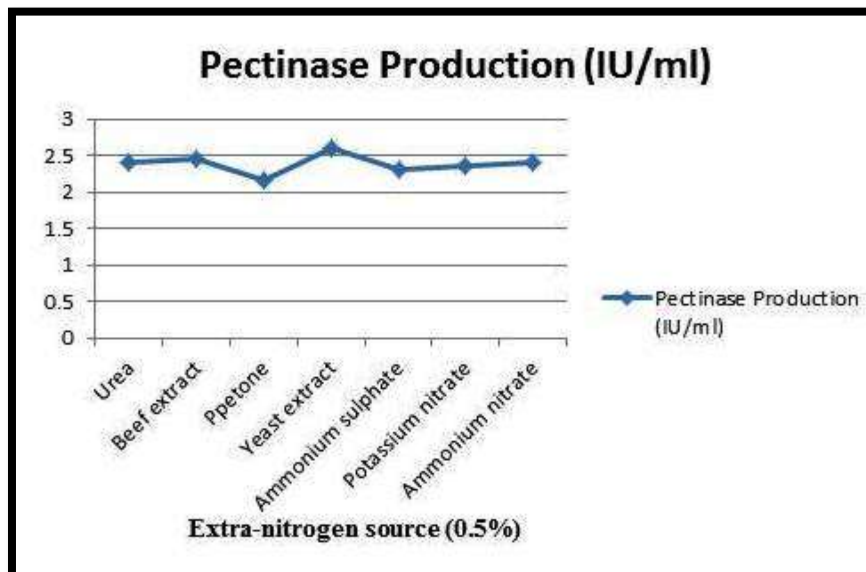


Figure 8: Effect of additional nitrogen source pectinase production

4. Conclusion

Identifying the significant factors affecting pectinase production using experimental design is essential for the production of enzymes on a large scale. *Aspergillus niger AG-1*, isolated from rhizospheric soil samples, showed highest pectinase activity, so it was explored for optimization of various process parameters affecting pectinase production by adopting a OVAT (One Variable At-a Time) design. Optimization studies revealed that pH and Temperature, incubation time and additional nitrogen source (yeast extract) had a significant role in pectinase production. It is clear from Pareto chart that parameters like Glucose, Dextrose, Ammonium sulphate and peptone have a lower impact on pectinase production, while Maltose has a highest impact on pectinase production by *Aspergillus niger AG-1*. Maximum pectinase production (2.54 IU/ml) was seen after 56 hours of incubation, at 35^{0}C (2.52 IU/ml), 2.55 IU/ml at pH 6.0 and 2.55 IU/ml at 2% salinity. From these reports it can be concluded that the potent fungal isolate *Aspergillus niger AG-1* can be effectively exploited for the large-scale pectinase production with some of fine tuning.

References

1. Du, J., Shao, Z., & Zhao, H. (2011). Engineering microbial factories for synthesis of value-added products. *Journal of Industrial Microbiology and Biotechnology*, *38*(8), 873- 890.
2. Banat, I. M., Makkar, R. S., & Cameotra, S. S. (2000). Potential commercial applications of microbial surfactants. *Applied microbiology and biotechnology*, *53*(5), 495-508.
3. KV, N. S. (2022). Applications of Microbes in Food Industry. In *Application of Microbes in Environmental and Microbial Biotechnology* (pp. 323-338). Springer, Singapore.
4. Demain, A. L. (1983). New applications of microbial products. *Science*, *219*(4585), 709- 714.
5. Hermann, T. (2003). Industrial production of amino acids by coryneform bacteria. *Journal of biotechnology*, *104*(1-3), 155-172.
6. Wendisch, V. F., Bott, M., & Eikmanns, B. J. (2006). Metabolic engineering of *Escherichia coli* and *Corynebacterium glutamicum* for biotechnological production of organic acids and amino acids. *Current opinion in microbiology*, *9*(3), 268-274.
7. Burkholder, P. R., & McVeigh, I. (1942). Synthesis of vitamins by intestinal bacteria. *Proceedings of the National Academy of Sciences of the United States ofAmerica*, *28*(7), 285.
8. Wang, X. N., Niu, M. T., Fan, J. X., Chen, Q. W., & Zhang, X. Z. (2021). Photoelectricbacteria enhance the in-situ production of tetrodotoxin for antitumor therapy. *NanoLetters*, *21*(10), 4270-4279.
9. Brahmachari, G., Demain, A. L., & Adrio, J. L. (Eds.). (2016). *Biotechnology of microbial enzymes: production, biocatalysis and Industrial applications*. Academic Press. Brinton CS, Dore WH, Wichmann HJ, Willaman JJ, Wilson CP. Definitions written by the committee on

nomenclature of pectin of the agriculture-food division. *J Am Chem Soc* 1927; 49:38–40.

10. Kataria, A., Sharma, R., Sharma, S., Singh, B., Kaur, G., & Yakubu, C. M. (2021). Recent applications of bio-engineering principles to modulate the functionality of proteins in food systems. *Trends in Food Science & Technology*, *113*, 54-65.
11. Bull AT, Goodfellow M, Slater JH. Biodiversity as a source of innovation in biotechnology. *Annu Rev Microbiol.* 1992; 46:219–252.
12. Fernandes, I. D. A. A., Pedro, A. C., Ribeiro, V. R., Bortolini, D. G., Ozaki, M. S. C., Maciel, G. M., & Haminiuk, C. W. I. (2020). Bacterial cellulose: From production optimization to new applications. *International Journal of Biological Macromolecules*, *164*, 2598-2611.
13. 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 a-amylase family. *Journal of biotechnology*, *94*(2), 137-155.
14. Singh, R., Mittal, A., Kumar, M., & Mehta, P. K. (2016). Microbial proteases in commercial applications. *J Pharm Chem Biol Sci*, *4*(3), 365-374.
15. Ismail, A. R., & Baek, K. H. (2020). Lipase immobilization with support materials, preparation techniques, and applications: Present and future aspects. *International Journal of Biological Macromolecules*, *163*, 1624-1639.
16. Polizeli, M. L. T. M., Rizzatti, A. C. S., Monti, R., Terenzi, H. F., Jorge, J. A., & Amorim, D. S. (2005). Xylanases from fungi: properties and industrial applications. *Applied microbiology and biotechnology*, *67*(5), 577-591.
17. Sharma, A., Tewari, R., Rana, S. S., Soni, R., & Soni, S. K. (2016). Cellulases: classification, methods of determination and industrial applications. *Applied Biochemistry and Biotechnology*, *179*(8), 1346-1380.
18. Singh, R. V., Sambyal, K., Negi, A., Sonwani, S., & Mahajan, R. (2021). Chitinases production: A robust enzyme and its industrial applications. *Biocatalysis and Biotransformation*, *39*(3), 161-189.
19. Kashyap DR, Vohra PK, Chopra S, Tewari R. Applications of pectinases in the commercial sector: a review. Bioresourc Technology 2001; 77:215-227
20. Hoondal G.S., Tiwari R.P., Tewari R., Dahiya N., Beg Q.K., (2002) Microbial alkaline pectinase and their industrial applications: a review, *Appl. Microbiol.Biotechnol.* 59:409- 418.
21. Ridley BL, O'Neill MA, Mohnen D (2001) Phytochemistry 57:929. doi:10.1016/S0031- 9422(01)00113-3
22. Perez, S., Rodríguez-Carvajal, M. A., & Doco, T. (2003). A complex plant cell wall polysaccharide: rhamnogalacturonan II. A structure in quest of a function. *Biochimie*, *85*(1-2), 109-121.
23. Sakai T, Sakamoto T, Hallaert J, Vandamme EJ. (1993). Pectin, pectinase and protopectinase: production, properties and applications. *Adv Appl Microbiol*; 39: 231–94.
24. Devi NA, Rao AGA. (1996). Fractionation, purification and preliminary characterization of polygalacturonases produced by *Aspergillus carbonarius*. *Enzyme Microb Technol*; 18: 59-65.
25. Naessens M, Vandamme EJ. (2003). Multiple forms of microbial enzymes. *Biotechnol Lett*, 25: 1119-24.
26. Aguillar, G. & Huitron, C. (1990). Constitutive exopectinase produced by Aspergillus sp. CH-Y-1043 on different carbon sources. *Biotechnology Letters* 12, 655±660.
27. Karbassi, A. & Vaughn, R.H. 1980 Puri®cation and properties of poygalacturonic acid trans-eliminase from Bacillus stearothermophilus. *Canadian Journal of Microbiology,* 26, 377±384
28. Gainvors, A. & Belarbi, A. (1993) Detection methods for polygalacturonase producing strains of *Saccharomyces cerevisae*. Yeast 10, 1311±1319.
29. Singh, R. V., Sambyal, K., Negi, A., Sonwani, S., & Mahajan, R. (2021). Chitinases production: A robust enzyme and its industrial applications. *Biocatalysis and Biotransformation*, *39*(3), 161-189.
30. Gummadi SN, Panda T. (2003). Purification and biochemical properties of microbial pectinases –a review. *Proc Biochem*, 38: 987-96.
31. Luh BS, Phaff HJ. (1951). Studies on polygalacturonase of certain yeasts. *Arch Biochem Biophys*, 33:212–27.

32. Gummadi, Sathyanarayana N., and T. Panda. (2003). Purification and Biochemical Properties of Microbial Pectinases—a Review. *Process Biochemistry* 38: 987–96. doi:10.1016/S0032-9592(02)00203-0
33. Marcus L, Barash I, Sneh B, Koltin Y, Finkler A. (1986). Purification and characterization of pectolytic enzymes produced by virulent and hypovirulent isolates of Rhizoctonia solani Kuhn. *Physiol Mol Plant Pathol*, 29:325–36
34. Sakai T, Takaoka A. (1984). Purification, crystallization and some properties of endo- polygalacturonase from Aureobasidium pullulans. *Agric Biol Chem,* 49:449–58.
35. De Lorenzo G, Salvi G, Degra L, D'Ovidio R, Cervone F. (1987). Introduction of extracellular polygalacturonases and its mRNA in the phytopathogenic fungus *Fusarium moniliforme. J. Gen. Microbiol*., 133:3365–73.
36. Torimiro, N., & Okonji, R. E. (2013). A comparative study of pectinolytic enzyme production by Bacillus species. *African Journal of Biotechnology*, *12*(46), 6498-6503.
37. Manachini M, Fortina C, Parini C. (1987). Purification and properties of an endopolygalacturonase produced by *Rhizopus stolonifer*. *Biotechnol Lett*, 9:219–24
38. Polizeli MLTM, Jorge JA, Terenzi HF. (1991). Pectinase production by *Neurospora crassa*: purification and biochemical characterization of extracellular polygalacturonase activity. *J. Gen. Microbiol*., 137:1815–23
39. Kumar SS, Palanivelu P. (1999). Purification and characterization of an exoplolygalacturonase from the thermophilic fungus, *Thermomyces lanuginosus*. *World J. Microbiol. Biotechnol.* 15:643–6.
40. Nagai M, Katsuragi T, Terashita T, Yoshikawa K, Sakai T. (2000). Purification and characterization of an endo-polygalacturonase from *Aspergillus awamori*. *Biosci Biotechnol Biochem.,* 64:1729–32.
41. Souza JVB, Silva TM, Maia MLS, Teixeira MFS. (2003). Screening of fungal strains for pectinolytic activity: endopolygalacturonase production by Peacilomyces clavisporus 2A.UMIDA.1. *Process Biochem.,* 4:455–8
42. Rodrigues-Palenzuela P, Burr TJ, Collmer A. (1991). Polygalacturonase is a virulence factor in *Agrobacterium tumifaciens biovar 3. J. Bacteriol.* 173:6547–52.
43. Nozaki K, Miyairi K, Hizumi S, Fukui Y, Okuno T. Novel exopolygalacturonases produced by Alternaria mali. *Biosci Biotechnol Biochem* 1997; 61:75–80.
44. Paloma¨ki T, Saarilahti HT. (1997). Isolation and characterization of new C-terminal substitution mutation affecting secretion of polygalacturonases in *Erwinia carotovora ssp*. *carotovora. FEBS Lett.* 400: 122–6.
45. Maceira FIG, Pietro AD, Roncero MIG. (1997). Purification and characterization of a novel exopolygalacturonase from *Fusarium oxysporum f. sp*. lycopersici. *FEMS Microbiol Lett,* 154:37–43.
46. Tierny Y, Bechet M, Joncquiert JC, Dubourguier HC, Guillaume JB. (1994). Molecular cloning and expression in Escherichia coli of genes encoding pectate lyase and pectin methylesterase activities from Bacteroides thetaiotaomicron. *J Microbiol,* 76:592– 602.
47. Huang Q, Allen C. (1997). An exo-poly-a-D-galacturonosidase, Peh B, is required for wild type virulence of Ralstonia solanacearum. *J Bacteriol,* 179:7369–78.
48. Koboyashi T, Higaki N, Yajima N, Suzumatsu A, Haghihara H, Kawai S, et al. (2001). Purification and properties of a galacturonic acidreleasing exopolygalacturonase from a strain of Bacillus. *Biosci Biotechnol Biochem,* 65:842–7
49. Sharma M, Rathore M, Sharma N, Dayama S, Jogpal B, Dashora K, Sharma A. Isolation of pectinolytic bacteria from rotten tomato. J Medical Dental Research. 2011;1(3):158–6
50. Priya V, Sashi V. (2014). Pectinase enzyme producing microorganisms. *Int J Sci Res Pub*. 4(3):1-4.
51. Mukesh Kumar DJ, Saranya GM, Suresh K, Andal Priyadharshini D, Rajakumar R, Kalaichelvan PT. (2012). Production and optimization of pectinase from *Bacillus sp. MFW7* us- ing cassava waste. *Asian J Plant Sci Res.* 2(3): 369–75.
52. Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of bacteriology*, *173*(2),

697-703.

53. Patil, N. P., & Chaudhari, B. L. (2010). Production and purification of pectinase by soil isolate Penicillium sp and search for better agro-residue for its SSF. *Recent Research in Science and Technology*, *2*(7), 36-42.
54. J. H. Holliman, *Pathology, Springer US*, New York, NY, USA, 3 edition, 2012.
55. N. Torimiro and R. E. Okonji, A comparative study of pectinolytic enzyme production by Bacillus species. *African Journal of Biotechnology*, vol. 12, pp. 6498–6503, 2013.
56. Ahmed, A., Khan, M. N., Ahmad, A., Khan, S. A., & Sohail, M. (2019). Optimization of pectinase production from geotrichum candidum AA15 using response surface methodology. *Pakistan J Bot*, *51*, 743-750.
57. Wang, X., Kan, G., Shi, C., Xie, Q., Ju, Y., Wang, R., & Ren, X. (2019). Purification and characterization of a novel wild-type a-amylase from Antarctic Sea ice bacterium *Pseudoalteromonas sp. M175. Protein expression and purification*, *164*, 105444.
58. Kalaichelvan, P. (2012). Production and optimization of pectinase from *Bacillus sp. MFW7* using cassava waste. *Asian Journal of Plant Science and Research*, *2*(3), 369-375.