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Development, production and characterization of amylase enzyme isolated from *Hay bacillus- MTCC441* by using solid state fermentation

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Abstract

The current study aimed to perform development, Production and characterization of Amylase enzyme which istoday's interest in the utilization of extracellular enzymatic activity in various microorganisms has increased due to the possibility of employing microbes as biological sources of economically feasible enzymes for industrial use. One of the most significant enzymes, alpha amylase (EC 3.2.1.1, 1, 4-D glucan glucanohydrolase), is employed in a variety of industrial processes, including those in the brewing, baking, textile, detergent, and paper sectors. -amylase can be highly helpful in related applications due to its low pH stability, raw starch digestibility, and usage of high concentrations of starch. Considering environmental concerns, it is crucial to continue developing bio sustainable and renewable resource technology. The present work was carried out to comparatively see the production of amylase in medium where different combinations of bio-wastes like banana peel, orange peel, apple peel, potato peel were used in powder form in the production media instead of starch.The highest enzyme activity was observed at pH 7.0, optimum temperature 70°C and highest enzyme concentration is found in orange peel substrate.

Keywords: Hey Bacillus, Amylase enzyme, solid state fermentation, Bio-waste

Full length article**Corresponding Author*: Dr. Sunil J. Aher*, email:suniljaher@gmail.com

1. Introduction

Amylases are a group of enzymes that hydrolyze starch. Many enzymes act on starch or on the oligosaccharides derived from them. Nineteen enzymes have been classified as belonging to the microbial amylase group: hydrolases (EC 3) such as α-amylase, β-amylase, glucoamylase, α-glucosidase, debranching enzymes and transferases (EC 2) such as CGTase, 4-α-glucanotransferase, and a branching enzyme. First, the catalytic properties of each of the 19 enzymes were described to clarify the differences among them and their microbial sources [1]. When agricultural or animal products are processed commercially, Bio-industrial wastes are produced. These wastes can be employed as raw materials for other industrial operations since their chemical makeup indicates that they are typically rich in carbohydrates, minerals, and proteins. These have the primary benefit of being plentiful and renewable. These wastes can be used as a solid support, carbon or nutrient source for the manufacture of a variety of chemicals that are of considerable value because the carbon sources, nutrients, and moisture included in such wastes provide favorable circumstances for the growth of microorganisms. Enzymes, ethanol, furfural, reducing sugars, protein and amino acids, secondary metabolites, carbohydrates, lipids, organic acids, surfactants, activated carbon, methane, degradable plastic composites, cosmetics, resins, drugs, foods and feeds,

biosorbents, biopesticides, promoters, fertilizer, and other random products are the main products the fact that can be produced [2-5]. This work aims to isolate and identify bacteria efficient in producing α-amylase enzyme via the morphological, physiological and molecular characteristics.

2. Materials and methods

Experimental studies were carried out by following methods and materials used [6-16].

2.1. Collection of Bio waste samples

From juice bars and residential sources, samples of bio-waste items, such as potato, orange, banana, and apple peels, were gathered. These peels were exposed to sunlight for 10 to 15 days to dry. Peels were ground into powder form using a grinder and subsequently sieved to obtain finer powder after being entirely dry and devoid of moisture. For later usage, the powder form of each sample was kept in airtight containers.

2.2. Screening of amylase producing fungal strain

Through the use of the serial dilution approach, bacillus colonies that produce amylase were isolated from soil samples. On starch agar medium, 50 l of diluted soil samples up to 10-5 dilutions were plated. Gram's iodine solution (for amylase) is applied to the culture's plates after seven days of incubation at 28°C; plates with the highest zone of Clearance were chosen for amylase production.

2.3. Preparation of production media

Production Media was comprised of (g/l); Peptone 5gm, NaCl 5gm, Beef extract 1.5gm, Yeast extracts 1.5gm and different combination of Bio waste in 1 L of distilled waterinstead of starch. Different Bio waste ratio in grams used in production media are as follows; Orange (1gm), Banana (1gm), Potato (1gm), Apple (1gm), Orange: Banana (1:1), Orange: Apple (1:1), Orange: Potato (1:1), Apple: Potato (1:1), Potato: Apple: Orange (1:1:1). Once 100 ml of media with each of the above Bio-waste combinations was prepared, the medium was autoclaved.

2.4. Crude enzyme extraction

We develop 100 ml of media, add various bio-waste combinations, and then inoculate the medium with a screened culture of Hay bacillus in a laminar airflow using an inoculation loop. For four days, the flasks were incubated at 30 °C. After 4 days, the production media in the flask was covered in a clear mat of Hay bacillus. After that, it was put through the Whatman filter paper.

2.5. Enzyme assay

The activity of extracellular amylase was followed by Bernfeld, 1955. 1 mL of crude enzyme from each combination was taken in separate test tubes and 1 ml of 1% starch solution, 3.5 ml of citrate phosphate buffer (pH 7.0) was added to it. The reaction mixture was incubated at 40^{o}C for 30 min. 2 ml of DNS reagent was added to all the test tubes and then kept in boiling water for 5 minutes, then cooled. The amount of reducing sugars in the final mixture was quantified by DNS method according to Miller

2.6. Study of kinetic parameters of alpha Amylase

Various kinetic parameters for fungal amylase were carried out such as effect of temperature, effect of pH, time of incubation and substrate concentration were studied.

2.6.1. Effect of temperature on amylase activity

Optimum temperature needed for amylase activity was estimated by incubating the reaction mixture at different temperatures from 4°C -100°C by dinitro salicylic acid method

2.6.2 Effect of pH on amylase activity

The effect of pH on enzyme activity was studied by performing the enzyme assay atdifferent pH using citrate phosphate buffer (pH range 3-11). The optimum pH of enzyme was determined by incubating the enzyme with different pH buffer as described above and assay was carried by dinitro salicylic acid method.

2.6.3 Effect of incubation time on enzyme activity

The effect of incubation time on the activity of amylase was studied by performing the assay at different time intervals from 10 min-40 min.

2.6.4 Effect of substrate concentration on enzyme activity

Optimum concentration needed for enzyme activity was carried out by incubating the reaction mixture for 30 min at different concentration of starch solution (1%-4%) by dinitro salicylic acid method.

2.6.5 Determination of reaction time and storage stability of crude amylase

To optimize the enzyme-substrate reaction time, the assay was carried out at different reaction times ranging from 10 min to 40 min in a water bath under optimum reaction temperature and pH.
To determine the storage stability of crude amylases, the enzyme solution was stored at room temperature for 8 days and amylase activity was measured after 2-day intervals.

3. Results and Discussions

3.1. Effect of different combination of Bio-waste on amylase production

To check the Bio-industrial wastes suitability for amylase production by Hay Bacillus- MTCC 441 species was done with the addition of different combination of Biowaste as carbon source. The detailed study explained in Table 1 and Figure 1.

3.2. Effect of temperature

For different incubation temperatures (30°C, 50°C, 70°C and 90°C), maximum enzyme activity was observed at 70°C.

Table 1: Enzyme Activity

Substrate Combination	Ratio (gm)	Enzyme Activity U/ml/min
Orange	1	18.058
Banana	1	25.4277
Apple	1	31.3350
Potato	1	29.3788
Orange-Apple	1:1	28.9183
Orange-Potato	1:1	36.4545
Orange-Banana	1:1	19.4647
Apple-Potato	1:1	25.1126
Potato-Orange-Apple	1:1:1	53.061

Table 2: Concentration of Substrate

Substrate Combination	Optimum Concentration
Orange	2%
Banana	1%
Apple	3%
Potato	4%
Orange-Apple	4%
Orange-Potato	1%
Orange-Banana	3%
Apple-Potato	4%
Potato-Orange-Apple	1%

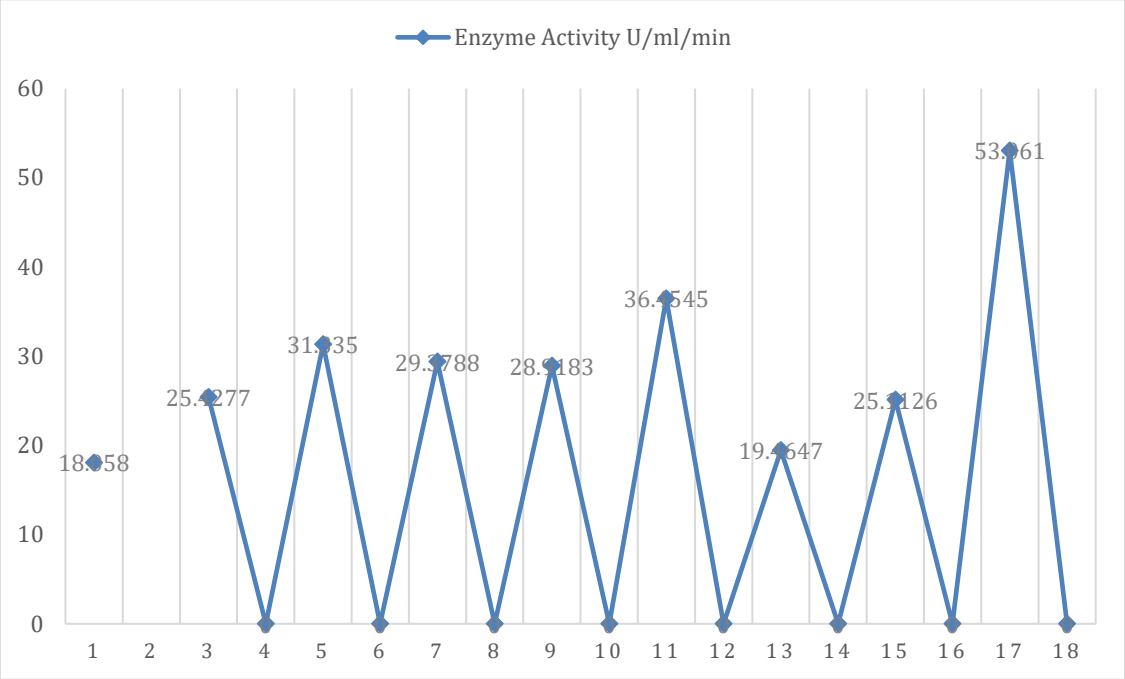

Figure 1: Effect of different combination of Bio-waste on amylase production

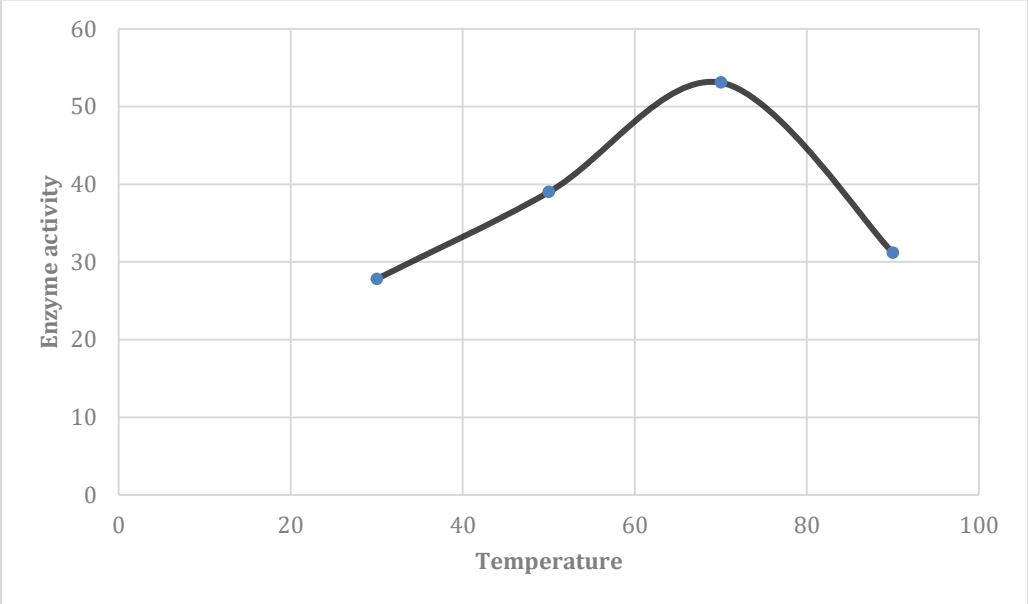

Figure 2: Effect of temperature on enzyme

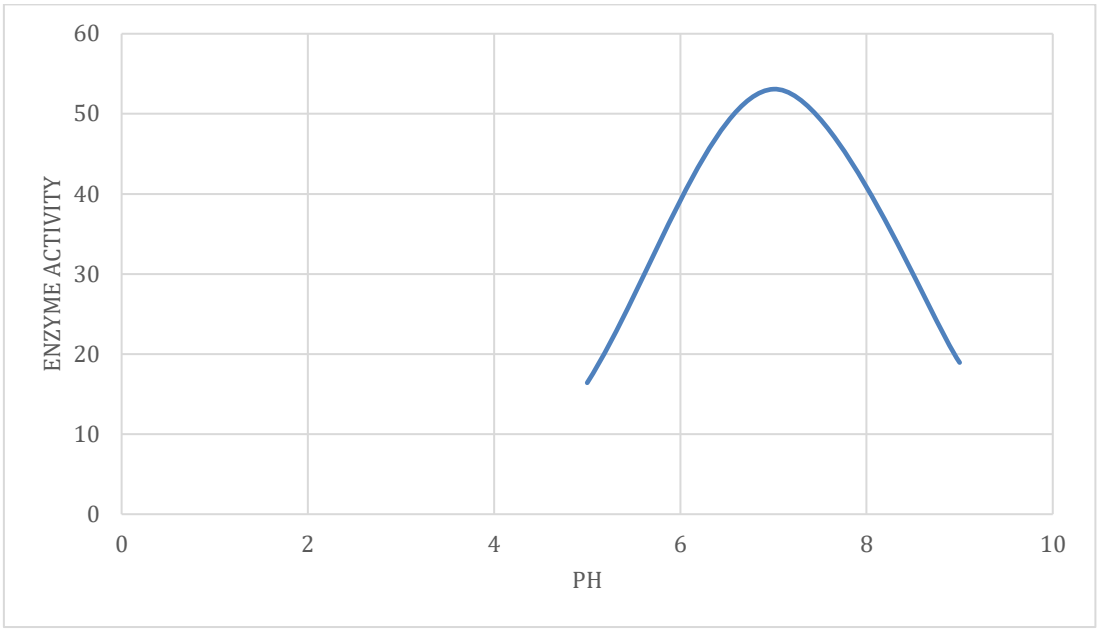

Figure 3: Effect of pH on enzyme

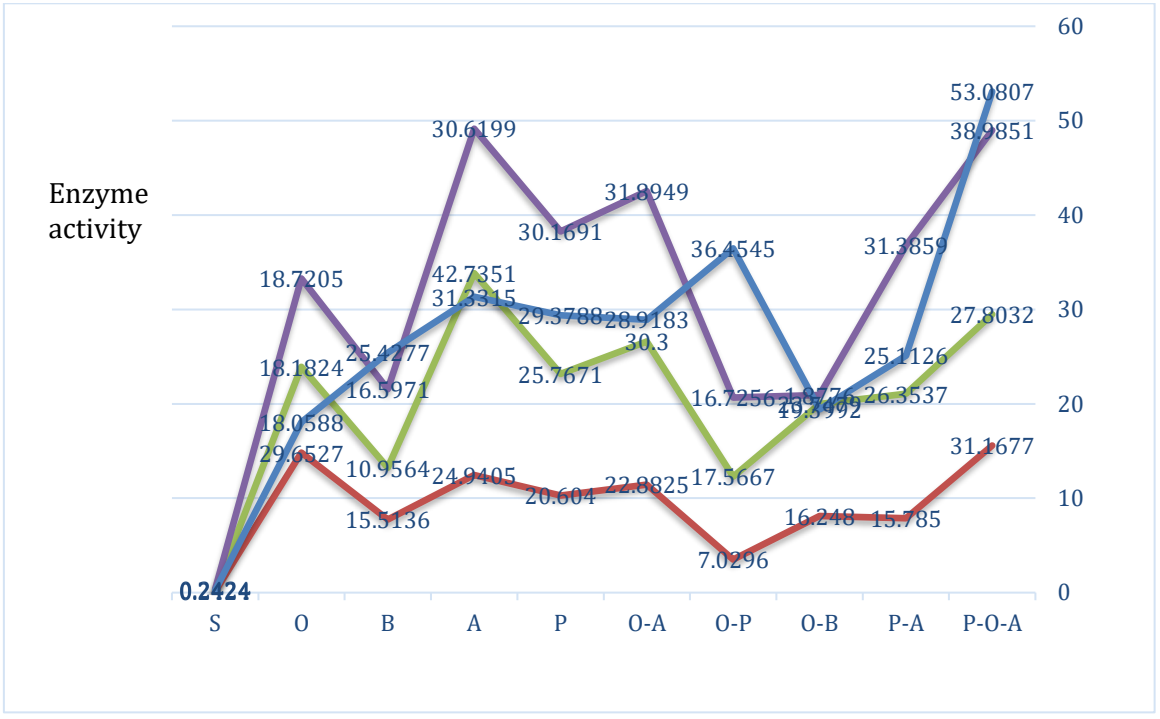

Figure 4: Effect of Substrate Concentration on enzyme activity

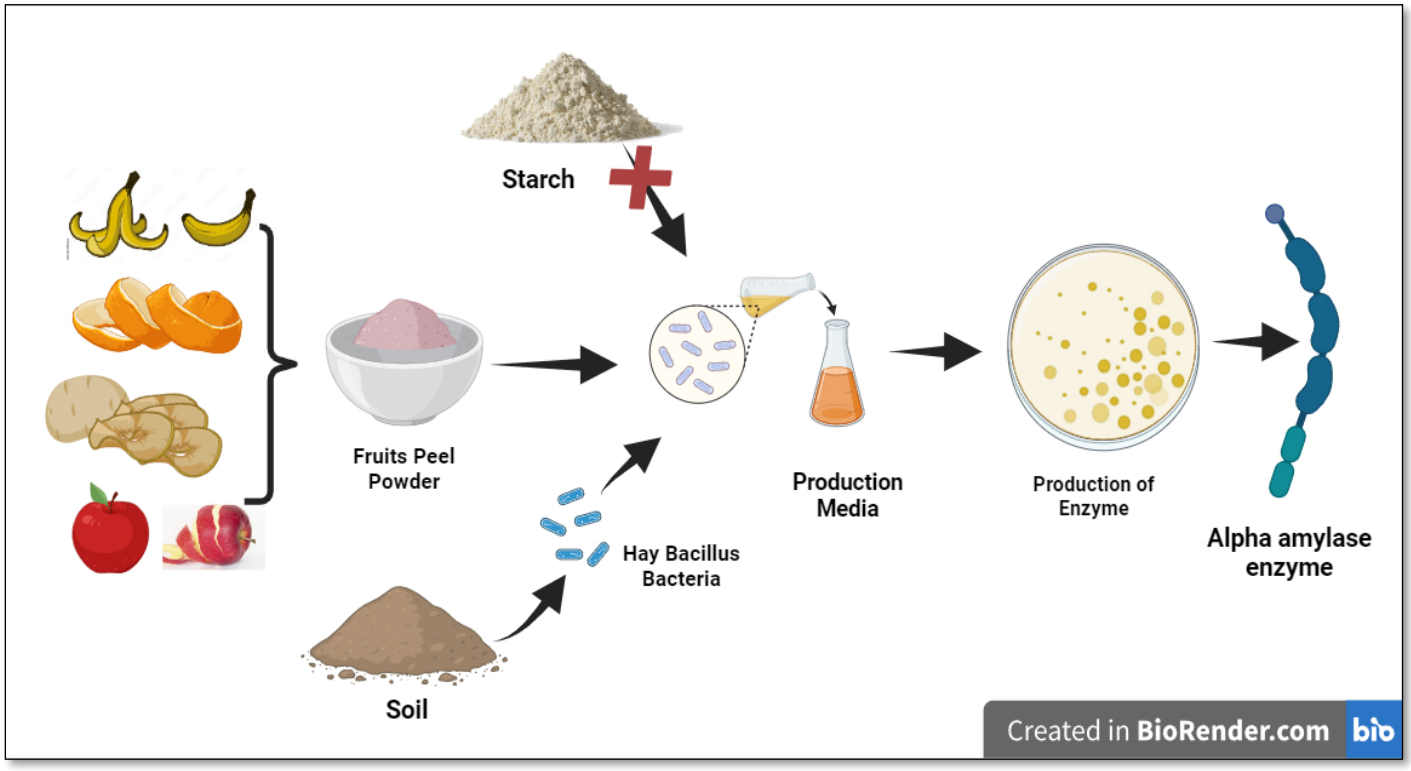

Figure 5: Graphical Abstract

When temperature was increased or decreased beyond this point, there was a decrease in enzyme activity. The temperature study explained results in Figure 2.

3.3. Effect of pH

For the selected Hay Bacillus- MTCC 441, highest enzyme activity was observed at pH 7.0 As pH increased, enzyme production also increased with the highest value at pH 7.0. But when the pH was increased further, enzyme production was found to be decreased as indicated in figure 3.

3.4. Effect of incubation time

The optimum incubation time for fungal amylase found to be 40 min.

3.5. Effect of substrate concentration

Amylase activity increased on increasing starch concentrations (1.0%, 2.0%, 3%, 4%). The % concentration were shown in Table 2. It was observed that enzyme activity increased linearly with the increase in substrate concentration. The optimum substrate concentration was found to be 4% as shown in figure4.

4. Conclusions

Hay bacillus- MTCC441 was found to be the best microorganism for the production of amylase enzyme from Bio waste materials. From the different substrates tested apple peel and combination of apple peel, potato peel and orange peel showed good efficiency as a substrate for high yield of α-amylase under solid state fermentation. *HAY BACILLUS- MTCC441*was able todegrade the starch when used as a substrate within a concentration of 1-4%. Optimum activity was observed at 4% starch concentration. Effects of pH on enzyme activity indicate the synthesis of amylase enzyme. This property makes the enzyme suitable for industrial production of paper and detergents. The enzyme activity increased progressively with increase in temperature from 30-70˚C and above 70˚C the enzyme activity gets decreased because of denaturation of enzyme protein. The enzyme retained 53% of its activity. Different temperature effect indicating thermostability of the enzyme. Optimum activity of the enzyme occurred at 70˚C

Conflict of interest

The authors declare no conflict of interest.

Author's contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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