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Study of Yeast Production of Pectinase Enzyme and the Effect of Different Physiological Conditions on Its Production

Badia Abdul Razzaq Malla Obaida
Department of Biology, College of Science, University of Mosul, IRAQ.

Corresponding Author: badia_jamal@yahoo.com

ABSTRACT

Pectinase enzymes are considered industrially important enzymes, and their importance is due to their use in many industries, such as extracting and liquefying fruit and vegetable juices, paper and textile industry, tea and coffee brewing, extracting oils with removing gum from plant fibers and treating factory wastes containing pectic substances. Microorganisms such as bacteria and fungi, including yeasts, produce pectinase enzymes on a commercial scale. Recently, it has been noticed that there has an increase in interest in its production of yeasts due to its characteristics, ease of development, and short time required for incubation.

Keywords- Yeasts, Pectinase enzymes, Production.

I. INTRODUCTION

Enzymes can be defined as protein substances that are secreted by living organisms and are specialized in specific biochemical processes, and they are found in all primitive and high-end organisms. Enzymes can be obtained industrially from three main sources: higher plants, animals and microorganisms. The production of enzymes from high-end plants or animals is not economically feasible, as it requires large areas to grow these plants or raise animals. Among the reasons why microorganisms are the best sources of enzymes are:

1. Microorganisms are rich in many enzymes, and by providing the appropriate conditions for the production of a specific enzyme, the microorganism can be stimulated to secrete this enzyme in a large amount.
2. Production speed due to the rapid reproduction rate of the microorganism.
3. Lower costs.
4. It does not need specific seasons, as is the case with plants that are grown according to the appropriate agricultural season (Al-Ani, 1993).

Enzymes can be divided into two groups:

• ***Exogenous enzymes:*** enzymes are secreted outside the cells in the growth medium. To extract the enzyme, a centrifugal process is carried out to get rid of the suspended cells, then the enzyme is separated from the clear solution by precipitation

• ***Endogenous enzymes:*** enzymes are found inside cells in the cytoplasm, and to obtain them, cells need to be crushed or analyzed to release the enzyme and then separate by the usual methods (Favela-Torres *et al*., 2006).

Bacterial compounds are polysaccharides formed in the middle plate of the plant cell wall in higher plants (Kavuthodi and Sebastian, 2018). The enzymes that break down these pectic compounds are known as pectinases or pectinolytic enzymes, which are high molecular weight acidic polysaccharides (Viverk *et al*., 2011). It is divided into two main groups: Pectin Methyl Esterase (PE), which removes methyl groups, and the enzyme plygalactouronase, which breaks the galactouronan chain (Garg *et al*., 2016; Oumer and Abate, 2018).

Types of Pectinase Enzyme

1. Pectinesterase (PE)
2. Polygalacturonases (PGases)
3. Pectatelyase (PGL)

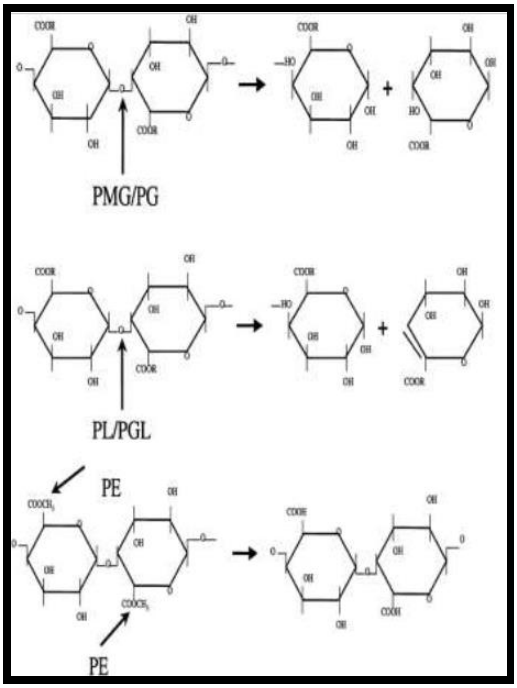

Figure 1: Types of pectinase enzyme (Mohnen, 2008)

Yeasts Produced Pectinase Enzyme

Attention was drawn to the production of pectinase enzymes produced from microorganisms as an alternative to animal and plant enzymes due to the ease of development and handling of these organisms, and there are many organisms that have the ability to produce this enzyme, including bacteria *Entero coccusasseliflavus*, *Staphylococcus aureus*, and *Staph. lentus* (Lateef *et al*., 2020), *Cloctridium spp*., *Bacillus spp.* and *molds*, *Alternaria alternate*, *Penicillium spp.* (Kashyap and Chandra, 2015; Mesfin and Wonltee, 2019; Hussein, 2019), *Aspergillus spp.* (Shokur and Haider, 2020), *Rhizoctonia solani*, *Rhizopus stolonifer*, *Mucor mucedo*, *Aureobasiduim pullulans*, *Neurospora rassa* and *Sclerotium rolfsii* (Kiran *et al*., 2014). As for the yeasts that produce the pectinase enzyme, they are clarified in Table No. (1):

Table 1: Yeasts produced of Pectinase Enzyme

Name of Yeats	Source
Saccharomyces cerevisiae Tokay ŁOCK 0204	(Wilkowska *et al*., 2020)
Kluyveromyces marxianus NCYC 179	(Wilkowska *et al*., 2020)
Metschnikowia sinensis 1.4 TKC	(Wilkowska *et al*., 2020)
Debaryomyces hansenii TEM-24	(Oskay and Yalçin, 2015)
S. fragilis	(Luh and Phaff, 1954)
Rhodotorula sp.	(Moyo, 2003)
Wickerhamomyces anomalus	(Martos *et al*., 2013)
Cryptococcus sp.	(Moyo, 2003)
Candida sp.	(Moyo, 2003)
Pichia pastoris NRRL-Y-7556	(Oskay and Yalçin, 2015)
Galactomyces geotrichum TEM-1	Oskay and Yalçin, 2015)
Saccharomyces cerevisiae Tokay ŁOCK 0204	(Wilkowska *et al*., 2020)
Kluyveromyces marxianus NCYC 179	(Wilkowska *et al*., 2020)

II. METHOD EXTRACTION OF PECTINASE ENZYME

These enzymes are commercially produced using solid-state fermentation and submerged culture methods (Kiran *et al*., 2014).

1. Solid-State Fermentation Method

In this method, wheat bran or defatted rice bran is used as a nutrient medium for mold development. Some food industry residues, such as the leftover materials from the process of squeezing apples, grapes and beet, encourage the production of the enzyme if they are present in the food medium, because they contain pectin, which stimulates the production of the enzyme. Some other components are added to the food medium such as some salts, acids or neutral solutions in order to regulate the acidity function during growth. The fermentation period takes about 7 days, but when using *A. niger* mold, the enzyme is produced within 36-72 hours. The bran containing the enzyme is dried after the fermentation period, which can be used directly in the form of a crude enzyme, or the enzyme is extracted from the bran using an appropriate solvent and the enzyme is concentrated under vacuum or by ultra-filtration. The enzyme powder, whether raw or pure, can be obtained by spray drying or precipitation using neutral salts or solvents.

2. Submerged Fermentation Method

The nutrient medium used in the production of pectinase by flooded culture method consists of 2% wheat bran, 2%, $4SO_2(NH_4)$, 25% KH_2PO_4, 0.25% yeast extract, 1% pectin and the pH is adjusted to 3.8 by hydrochloric acid and a small amount of silicon is added to prevent foam. The fermentation process begins after inoculation of the nutrient medium with spores, and aeration and stirring are used during the fermentation process.

The fermentation process takes about 72 hours and the spores or mycelium are separated by filtration and the filtrate is cooled to 0-1 ^{o}C. Then the enzyme was precipitated by adding $4SO_2(NH_4)$ over a period of 4 hours. The precipitate is washed after leaving it for about 12 hours and then dried to obtain the enzyme powder. Figure (2) shows the steps for producing pectinases.

The amount of resulting enzyme is less in the case of using submerged cultures and the enzyme composition is not homogeneous compared to the solid-state fermentation method (Al-Ani, 1993) which is characterized by its high productivity, low cost as well as its simple requirements (Hölker and Lenz, 2005).

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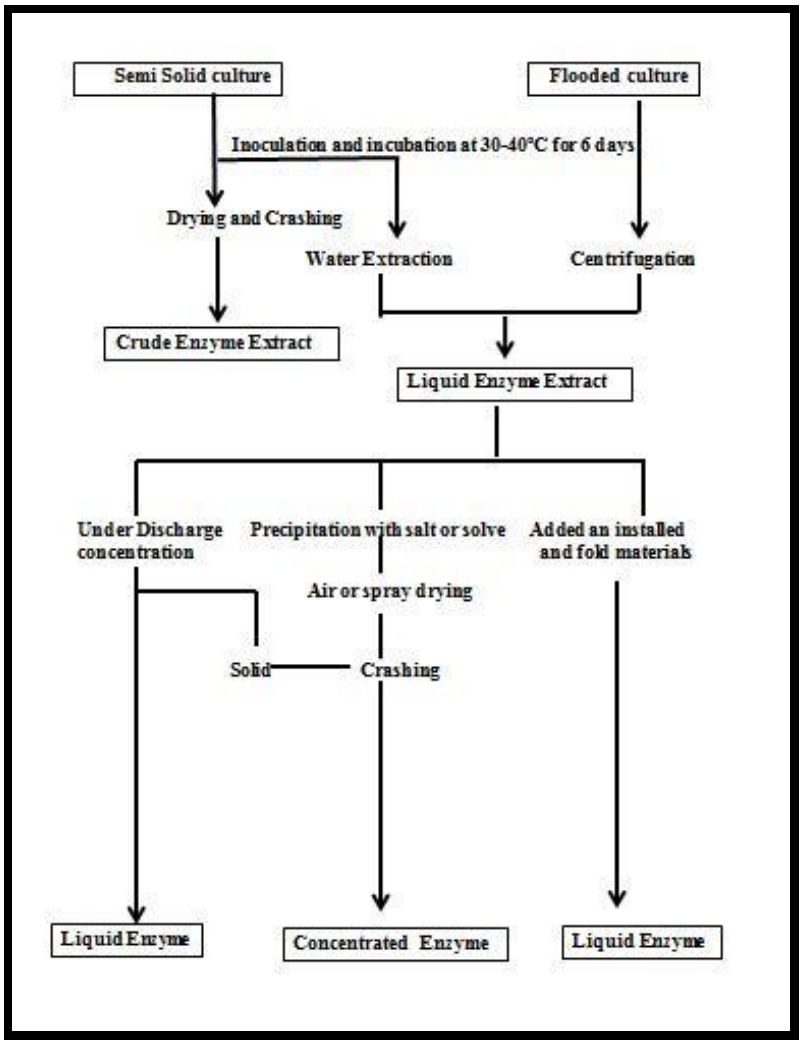

Figure 2: Steps of Pectinase Enzyme Production from Yeasts (Al-Ani, 1993)

III. FACTORS AFFECTING THE PRODUCTION OF PECTINASE ENZYME

The production of pectinase enzyme is affected by many factors, including carbon source, nitrogen source, pH function, incubation period, temperature and size of the vaccine used (Murad and Foda, 1992).

1. Carbon Sources

The carbon source affects the production of pectinase enzyme, as it was observed that the use of glucose as a carbon source at a concentration of 2% + 1.8 pectin led to an increase in production of pectinase enzyme compared to the amount of enzyme produced when glucose was used at a concentration of 1% in the yeast *C. albidus* (Federici, 1985), *G. lactis* (Pardo et al., 1991), *K. fragilis* (Garibay-Garcia et al., 1987).

2. Nitrogen Sources

One of the most important factors affecting production efficiency and quality is the nitrogen source, and organic nitrogen sources such as yeast extract and peptone are better than inorganic nitrogen sources.

3. pH

The optimum pH number for achieving maximum productivity is 5 (Murad and Foda, 1992).

4. Incubation Period

The incubation period is considered one of the factors affecting the production, and the 3 days of incubation is appropriate for the growth of most types of yeasts and for obtaining optimal productivity (Federici, 1985; Blanco *et al*., 1999; Murad and Foda, 1992).

5. Inoculation

Pectinase production is related to the amount of dissolved oxygen in the food media. It was found that many yeasts increase their ability to produce pectinase enzyme when grown in anaerobic conditions that are completely unproductive when aeration is available, such as *K. marxianus* (Schwan and Rose, 1994) *K. fragilis* and *K. lactis* (Garcia-Garibay *et al*., 1987).

6. Inoculum size

Enzyme production is affected by the amount of inoculum used in the nutrient medium, and the best amount of inoculum to obtain a high yield of the enzyme is 4% vol (Murad and Foda, 1992).

IV. THE USES OF PECTINASE ENZYME

Pectin-degrading enzymes are used in many food and industrial fields, and the following are their most important uses (Oumer, 2017):

1. Purification of juices:

Pectinase enzymes reduce the amount of pectin present and reduce the viscosity of juice, which leads to acceleration of the filtration process and increase of juice clarity (Rai *et al*., 2004).

3. In the field of biotechnology (isolation of cell protoplasts):

Works to break down pectic compounds and obtain protoplasts.

4. *Winemaking:*

Adding pectinase enzymes during fruit crushing leads to reduced juice viscosity and smoother juice (Roldán *et al*., 2010).

5. Paper industry:

Pectinase enzymes in the paper industry work to dissolve depolymerisepectins and reduce the amount of pectin in the solution and increase filtration and this leads to an increase in paper whiteness (Reid and Ricard, 2000).

6. Removing Gum from Plant Fibers:

Pectinase enzymes work on degrading the gums of plant fibers when used in the textile industry.

7. Cotton Textile Processing:

Pectinase enzymes conjugate with amylases, lipases, cellulases and hemi-cellulases and remove them in a safe manner without affecting the product (Hoondal *et al*., 2002).

8. Wastewater treatment:

Wastewater contains pectic compounds that microorganism such as yeasts produce pectinase enzymes and thus degrade and remove them from the water in a safe manner (Beg *et al*., 2000).

9. Animal feed:

Pectinase enzymes are used in the production of animal feed, as they decompose the non-biodegradable fibers and reduce the viscosity of the feed, which helps to increase the absorption of nutrients (Jayani and Gupta, 2005).

10. Oil extraction:

It is used in oil extraction, such as extracting oils from citrus peels, as it decomposes pectic compounds (Mohnen, 2008).

11. Purification from viruses:

Pectinase enzymes purify phloem tissues from viruses (Reid and Ricard, 2000).

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