Studies in isolation, characterization and applications of microbial alkaline protease enzyme

Dissertation-1 Report

Submitted by

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CERTIFICATE

This is to certify that Mayuri Sharma has personally completed M.Sc. Pre-dissertation entitled, **"Studies in isolation, characterization and applications of microbial alkaline protease enzyme"** under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study. No part of Dissertation-1 Report has ever been submitted for any other purpose at any University.

The project report is appropriate for the submission and the partial fulfilment of the conditions for the evaluation leading to the award of Master of Food Technology.

Signature of Supervisor

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DECLARATION

I hereby declare that the work presented in the pre- dissertation report entitled "Studies on isolation and characterization of alkaline protease enzyme" is my own and original. The work has been carried out by me at School of Agriculture, Lovely Professional University, Phagwara, Punjab, India under the guidance of Dr. Yogesh Gat**,** Assistant Professor (Food Technology) of School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree of Master of Science in Food Technology.

Date: 12-05-2018 **Mayuri Sharma**

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I certified that the above statement made by the student is correct to the best of my knowledge and belief.

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CHAPTER 1 INTRODUCTION

Enzymes are biocatalysts which are essential for life as they catalyze almost every biological process (Hinnemann and Norskov, 2006). Enzymes since ancient times have been used in the manufacturing of different food products like beer, wine, vinegar, cheese, sour dough and in the production of commodities like leather, linen and indigo (Kirk *et al.,* 2002). Proteolytic enzymes are present in all living organisms and helps in cell growth and differentiation. Proteases are the hydrolytic enzymes that act as a biocatalyst for the cleavage of proteins into smaller peptides and amino acids. Proteases are very important enzymes used in industry accounting for more than 60% of total global enzyme sale (Ningthoujam *et al.,* 2009).

According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, proteases are classified in group 3 (hydrolases) and sub group 4 (that hydrolyse the peptide bonds) (Sumantha et al., 2006). Protease can be subdivided into two major groups that are endopeptidases (cleave internal peptide bonds) and exopeptidases (cleave C- or N- terminal peptide bonds). Endopeptidases can be further classified into four major groups. This includes serine proteases (EC.3.4.21), cysteine proteases (EC.3.4.22), aspartic proteases (EC.3.4.23) and metalloproteases (EC.3.4.24). Serine proteases include serine alkaline proteases and subtilisins (Rao *et al.,* 1998). Alkaline proteases are those proteases which are active from neutral to alkaline pH range. Alkaline protease can be obtained from various sources like fungi, bacteria and insects. Alkaline protease is produced by most of alkalophilic microorganisms however; interest is mainly focused on those which yield high amounts of enzyme.

CHAPTER 2 PROBLEM BACKGROUND

To the best of our knowledge enough literature is available on alkaline protease production, purification and its characterization. With continuous increase in demand of alkaline protease, there is a need of exploring information in broader way to increase the commercial production from newly isolated strains. Alkaline protease is widely used in various industrial sectors like detergent, leather, silver recovery etc. However, its application in food industry have not been much exploited. Alkaline protease enzyme can be used in the production of hydrolysates which can be further used to produce value added products.

3.1 Isolation of alkaline protease producing microorganisms

The alkaline producing microorganisms are isolated from various sources by surface plating on an alkaline medium and subsequent screening for the desired characteristics. The different sources of alkaline protease are soil, sugar molasses, water, degraded feather, waste water, marine sediments, compost, thai fish sauce etc (Sharma *et al.,* 2016).

3.2 Production of alkaline protease by submerged fermentation

Fermentation is a metabolic process that converts complex substrates into simpler substances by the action of various microorganisms like bacteria and fungi. In submerged fermentation free moving liquid substrates are utilized like broth and molasses. The substrate is used up quite rapidly hence constant supplementation of nutrients is required (Subramaniyam *et al.,* 2012). The production of enzyme and different aspects of microbial growth are affected by the nature of fermentation, solid or submerged. (Hamzah *et al.,* 2009). The submerged culture has an advantage of easy sterilization and it is easier to control the processes in these systems (Vidyalakshmi *et al.,* 2009). Proteases are generally produced using submerged fermentation due to its apparent advantages in consistent enzyme production characteristics with defined medium and process conditions and advantages in downstream in spite of the cost-intensiveness for medium components (Prakasham *et al.,* 2005).

3.3 Production of alkaline protease by solid state fermentation

Solid state fermentation is described as a fermentation process that requires a solid substrate with absence or near absence of free water; however, it should posses enough water to promote the growth of microorganisms (Singhania *et al.,* 2009). The substrate serves as a source of nutrients and also helps in the attachment of microbial cells. It is an eco- friendly technique because it utilizes solid agro - industrial residues as a solid matrix (Thomas *et al.,* 2013). It is well established that in various cases like enzymes, bioactive compounds, etc. the products titres formed in submerged fermentation are very lesser than submerged fermentation (Pandey *et al.,* 2000). Other advantages of submerged fermentation are less waste water produced, low energy requirement and lesser risk of bacterial infection (Thomas *et al.,* 2013). However, there are limitations of solid state fermentation like it is relatively slower than submerged fermentation

because bulk solid forms an additional barrier. Different microorganisms can grow in submerged fermentation but only fungi can grow up to a significant level in near absence of water (Raghavarao *et al.,* 2003). The extracellular alkaline protease produced by *Bacterial subtilis* isolate showed 45% higher production in solid state fermentation than in submerged fermentation (Soares *et al.,* 2005).

3.4 Applications of alkaline protease

Microbial alkaline protease has numerous applications in different industrial sectors like leather, detergent, food, clinical, etc. Many industries have launched different products which are hinged on alkaline protease enzymes. In food industry alkaline protease plays an important role in the formation of value added products. Alkaline protease also plays a significant role in waste management. The applications of alkaline protease enzyme are described subsequently.

3.4.1 Detergent industry

Enzymes are of great interest to the detergent industry because of their ability to remove a large variety of stains. The alkaliphilic enzyme has a major applications in detergent industry. The detergent industry uses several hydrolytic enzymes that work in the alkaline pH range. Apart from being used in laundry detergents the alkaline protease enzyme is also used in household dish washing detergents and also in the formulation of institutional and industrial cleaning detergents (Showell 1999). Enzymes can be used as a detergent additive if it possesses two qualities firstly an alkaline pH range and secondly compatibility with the detergents.

3.4.2 Leather industry

The alkaline protease enzyme has an extensive application in the leather industry. Different steps like soaking, dehairing, bating and tanning are involved in leather processing. Traditionally leather processing involved the use of chemicals such as sodium sulfide and lime. These chemicals are hazardous as well as expensive. The uses of these chemicals are not considered as ecofriendly as the problems are encountered with the effluent disposal. An alternative to the conventional method is the use of enzymes, which have led to improvement in the leather quality and decrease in environmental pollution. protease enzyme from certain bacterial species have proved to be efficient in the hair removal process. Proteases play an important role in the

removal of non fibrillar proteins like albumins and globulins. They also cause selective hydrolysis of non-collagenous proteins

3.4.3 Food industry

Alkaline proteases have been used in the preparation of protein hydrolysates of high nutritional value. The protein hydrolysates play an important role in blood pressure regulation and are used in infant food formulations, specific therapeutic dietary products and the fortification of fruit juices and soft drinks (Neklyudov et al. 2000).

CHAPTER 4 RESEARCH GAP

There are several reports about the isolation of microbial protease enzyme. According to our knowledge very few researchers have given focus on isolation and characterization of alkaline protease enzyme from natural resources. As well there is less emphasis laid on the applications of microbial protease enzyme in food industry.

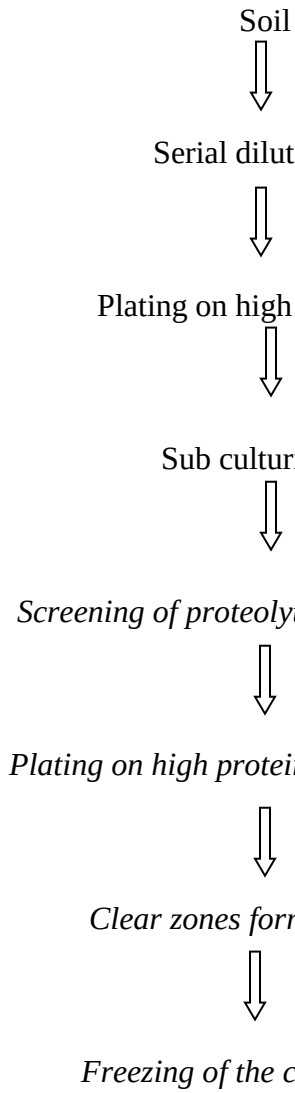
CHAPTTER 5 OBJECTIVES

1. Isolation and purification of microbial alkaline protease enzyme.
2. Optimization of process parameters for the production of alkaline protease.
3. Characterization of alkaline protease enzyme.
4. Application of isolated purified protease enzyme in suitable system.

CHAPTER 6. METHODOLOGY

*6.1 Objective one***:** Isolation and purification of microbial alkaline protease enzyme.

Isolation will be brought about by the following method



*6.2 Objective two***:** Optimization of process parameters for the production of alkaline protease.

- The production of protease is carried out by fermentation process as per the standard meathod by Rao and Narasu 2016. The important consideradions during fermentation process are temperature and pH.
- The second step involves centrifugation of the broth to get purified enzyme

6.3 Objective three: Characterization of alkaline protease enzyme.

- The effect of pH on the enzyme is determined by incubating microorganism in different buffer solutions.

pH(buffer solutions)	Alkaline protease activity
4	
5	
6	
7	

- The effect of temperature is determined by growing microbes in different temperature conditions.

Temperature	Alkaline protease activity
30°C	
40°C	
50°C	
60°C	

- Different metal ions and protease inhibitors are added in the media to check its effect on production of protease enzyme and to determine the type of protease produced

6.4 Objective four: Application of isolated purified protease enzyme in suitable system.

- For the production of whey hydrolysates whey protein concentrate is incubated with alkaline protease according to the standard method (Sinha *et al.*,2007).

CHAPTER 7 EXPECTED RESEARCH OUTCOMES

- Isolation of alkaline protease from natural sources
- Standardizing the process for scale- up and production of alkaline protease
- Application of alkaline protease in suitable system

CHAPTER 8 CONCLUSION

Alkaline proteases are highly significant enzymes considering their stability and activity in the alkaline pH range. Microbes are an excellent source of protease enzyme considering their ability of rapid growth in limited space, relatively cheap source and accessibility to genetic engineering. The applications of microbial alkaline protease in different industrial sectors are also discussed. Alkaline protease has an extensive use in detergent and leather industry. Many of the researches have been done on establishing compatible enzymes for these sectors. However, more research is needed for the application of microbial alkaline protease especially in the case of food industries because alkaline protease from microbial sources is a relatively inexpensive method of producing different value-added products.

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