

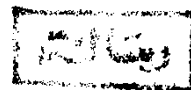
FACTORS AFFECTING THE PRODUCTION OF PROTEINASE
FROM AEROBIC SPORE-FORMERS

Thesis
Submitted in Partial Fulfilment of
the Requirements for the Degree of
Master of Science
in Agricultural Bacteriology

By

RAWIA FATHI GAMAL

B. Sc. (Agric.), Ain Shams Univ., 1967



4797



Ain Shams University
Faculty of Agriculture

1971

547.755
R.F.

APPROVAL SHEET

Title of Thesis : Factors affecting the production
of proteinase from aerobic spore-
formers.

Name of Candidate : Rawia Pathi Gamal

Thesis Approved by :

S. A. ZAKY
M. Elmaghrabi
S. Elmaghrabi

Date: / /1971



ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Professor M.M. Taha for his deep interest and his supervision throughout this work.

I am also deeply indebted to Professor S.A.Z. Mahmoud, Head of Agric. Bacteriological Dept., Faculty of Agriculture, Ain-Shams University, for his kind supervision, instructive suggestions and his encouragement throughout the whole work.

I am as well grateful to Dr. R.M. Attia, Ass. Prof. of the Microbiological Research Division, Agric. Research Centre, for his valuable guidance, sincere helpfulness and supervision throughout the whole work.

Thanks are extended for the whole hearted cooperation to all my colleagues in the Agricultural Bacteriological Dept., and all those who have helped in the execution of this work.

Samia Pathi Carol.

1971.

INTRODUCTION.	1
REVIEW OF LITERATURE	1
MATERIALS	30
Bacteriological procedures.	31
Isolation of aerobic sporeformers	31
Identification of aerobic sporeformer	31
Maintenance of cultures	33
Starter	34
Fermentation	34
Enzyme recovery	34
Media used	35
Chemical procedures	37
Protease activity	37
Screening method	37
Gross Fuld method	37
Lohlein Volhard method	38
Determination of pH	39
Determination of moisture	39
Determination of ash	39
Buffers	40
Determination of nitrogen	40
Detection of amino acids by paper chromatography	41
Detection of sulfur by spot test	43
Detection of phosphorus by spot test	44
Detection of ferric ions by spot test	45
Detection of reducing carbohydrate by spot test	45
Detection of alpha amino carboxylic acid by spot test.	46
Detection of manganese, magnesium, zinc and calcium by spot test.	46

Introduction	
Chapter I. The isolation and characterization of the bacterial strain possessing the protease gene	
Factors affecting influencing bacterial proteinase production.	54
Effect of carbon sources	55
Effect of carbon concentration	56
Utilization of agricultural by-products and wastes for proteinase production.	58
Effect of nitrogen	62
Effect of pH's	67
Effect of buffers	72
Effect of phosphate concentration	75
Effect of activators	76
Effect of trace elements	80
Effect of incubation period	83
Effect of aeration	86
Extraction and fractionation method for bacterial proteinase.	88
Chemical analysis of pure proteinase	91
DISCUSSION	96
SUMMARY	112
BIBLIOGRAPHY	117
ARABIC SUMMARY	—

INTRODUCTION

INTRODUCTION

Enzymes are organic catalysts produced by all living cells, which govern the chemical reactions for life processes. Once elaborated by a cell, an enzyme can act independently of the cell if proper conditions are maintained.

In referring to production of enzymes, one is thus, dealing with the manipulation of living cells to obtain the maximum yield of the desired enzyme. Once synthesized by the cell, the enzyme may then be subjected to a variety of biochemical procedures for its recovery and purification in a diversity of forms suitable for many applications of economic importance.

Bacterial proteases are group of hydrolytic enzymes, overshadows all others in industrial significance and utilization. Bacterial proteolytic enzymes have the greatest commercial applications, especially in the field of technology, because the great part of the published work is of a fundamental nature and the companies interested in producing and marketing enzyme products, seldomly except their techniques except for general principles or the mentioning of some patents. At present, bacterial proteases are extensively used in many industries such as

carcass blanching, spot removing, fat rendering, tanning, skin, leather, silk and wool industries, pharmaceutical products and in medical treatment of burns and ulcers.

In recent years, it has been increasingly demonstrated that bacterial proteases can be used to good purpose in furthering numerous industrial processes. Leather constitute one of the largest and most diversified industries. The leather industry in A.R.E. consumed high quantities of animal skins, in producing about 20 million sq. feet of light leather and 5 million sq. feet of heavy leather. Bacterial proteases is extensively used in bating either in alkali or acid media. It is used ⁱⁿ bating box calf, clothing, softy, fancy gloving and all types of leathers.

In the A.R.E. high quantities of bacterial proteases of Bacillus subtilis origin are imported from foreign countries, such as France, Germany, and Denmark, amounting to approximately one thousand tons per annum. The production of this enzyme in Egypt will evidently save more than 100,000 pounds in foreign currency.

Therefore, it was found valuable to study its production from different raw materials using highly active locally isolated Bacillus subtilis.

Investigations were also focused on nutritional and environmental factors for maximum production of proteinase. The extraction, purification and chemical composition of an enzyme were also investigated.

REVIEW OF LITERATURE

4.1.1.1.1

The application of enzymes to the production of leather appeared from the very early observation that animal feces applied to the flesh side of hide, resulted in removal of non collagenous material and the softening of the leather. Hundreds of years passed before the active ingredients in the excreta were identified as proteases, hence making it possible to substitute pancreatic or mold enzymes for the bating of hides, (Gustavson, 1956).

From these early beginnings in fermentation, cheese making, and the tanning of leather, the application of enzymes has spread to the textile (desizing), paper (starch modification) industries as well as to nutrition, foods, also to pharmaceuticals and medicine.

Applications of bacterial proteolytic enzymes :

With few exceptions, enzymes which have found industrial application are hydrolytic. This is primarily because they are much simpler to deal with and historically have been easier to prepare.

1- Tanning of leather :

It was common practice to clean hides free of flesh and noncollagenous material by shearing them with a knife. It was a long time before the active ingredients were identified as proteolytic enzymes from the pancreas. For economic reasons, microbial proteases are used to-day for the bating of hides instead of a pancreatic powder. Enzymic treatment of hides is an indispensable step in the preparation of pliable and attractive leather of fine grade (Gustavson, 1956).

2- Food Industry :

The application of proteases to the food industry is rapidly becoming one of their major uses. Pure proteins are quite tasteless while some of the amino acids and certain peptide produced by protein hydrolysis are highly flavorful. Hence treatment with proteases of many foods rich in proteins may be expected to increase their flavour. Even more important are softening, tenderizing, increased digestibility, increased solubility and shortened cooking time.

For economic reasons, B. subtilis enzymes have been cleared for food use. The largest food use of enzyme

ion
purification/ of a solution in which
the solution is chilled.

3- Food Industry :

A relatively new application of proteases is to the treatment of stock feeds. For example enzymic attack on barley can improve its nutritional value in poultry feed. Similarly the enzymic digestion of whole fish powder can increase the nutritional value of animal feeds.

4- Baking Industry :

It is sometimes difficult to provide flour of sufficiently low protein content for the manufacture of biscuits. As a consequence, the doughs tend to become elastic and difficult to work. These difficulties can be remedied to a large extent by the addition of bacterial proteases.

5- Enzymatic Laundering :

By using bacterial enzymes when laundering heavily soiled clothes, on which protein containing substances such as blood and mucus form a considerable part of the

ent, the silk is hydrolyzed and the silk becomes readily soluble.

6- Silk industry :

The fibres in raw silk are surrounded by layer of silk gelatin, sericin, which is a protein. The sericin can be removed by treatment with bacterial proteases, which does not affect the silk fibres consisting of keratin.

7- Medical treatment of burns and ulcers :

Bacterial proteases have been tried on burns and ulcers with variable success, (Spier, Rees and Clifton, 1956).

8- Anti-inflammatory agents :

Inflammation in various parts of the body can often be relieved by anti-inflammatory agents which are mainly proteolytic enzymes, (Anonymous, 1962).