

**A  
STUDY PROJECT  
ON  
SCREENING AND ISOLATION & IDENTIFICATION OF  
AMYLASE PRODUCING SOIL BACTERIA AND  
HALOPHILIC BACTERIA**

**BY  
III B.SC., (M.Z.C)  
K. SRUJANA  
D. NAVEENA  
A. VENKATA LAKSHMI  
P. ANUSHA  
G. REVATHI  
2016-2017**

**UNDER THE GUIDANCE OF  
N. PRAVEENA KUMARI  
LECTURER IN MICROBIOLOGY  
Y.A.GOV'T. DEGREE COLLEGE FOR WOMEN,  
CHIRALA**



**Govt. of  
Andhrapradesh**



**NAAC-B**

## Introduction

Starch is the primary source of stored energy in cereal grains. Although the amount of starch contained in grains varies, it is generally between 60 and 75% of the weight of the grain and provides 70–80% of the calories consumed by humans worldwide. In addition to their nutritive value, starches and modified starches can be used to affect the physical properties of many foods. For example, commercial starches obtained from corn, wheat, various rices, and tubers such as potato, sweet potato, and cassava (tapioca starch) can be used in gelling, thickening, adhesion, moisture-retention, stabilizing, texturizing, and antistaling applications. Starch and products derived from starch are also important in the paper and textile industries. The unique chemical and physical characteristics of starch set it apart from all other carbohydrates.

Starch is a major carbohydrate reserve in plants. Variations in the extent of starch hydrolysis create a range of important products with different physical and chemical properties for food and pharmaceuticals. Starch consists primarily of D-glucopyranose polymers linked together by  $\alpha$ -1,4 and  $\alpha$ -1,6 glycosidic bonds. In forming these bonds, carbon number 1 (C1) on a D-glucopyranose molecule reacts with carbon number 4 (C4) or carbon number 6 (C6) from an adjacent D-glucopyranose molecule. Because the aldehyde group on one end of a starch polymer is always free, starch polymers always have one reducing end. The other end of the polymer is called the nonreducing end. Depending on the number of polymeric branches present in a starch molecule, there could be a large number of nonreducing ends. The glycosidic linkages in starch are in the alpha ( $\alpha$ ) configuration. Formation of an  $\alpha$  linkage is determined by the basic Carbohydrate Chemistry. Starch Polymer consists of Amylose and Amylopectin Amylose. Constituents of the Starch Granule 1 D-Glucopyranose—The ring form of the monosaccharide D-glucose. Reducing sugar—A monosaccharide, disaccharide, oligosaccharide, or related product

capable of reducing an oxidizing ion. A common test for the measurement of reducing sugars involves the reduction of cupric ions ( $\text{Cu}^{+2}$ ) to cuprous ions ( $\text{Cu}^{+}$ ). Glycosidic bond—Covalent linkage formed between D-glucopyranose units.

To illustrate the significance of the  $\alpha$  linkage, starch is sometimes compared to cellulose, a glucose polymer with  $\beta$ -1,4 bonds between subunits. This seemingly trivial difference results in large differences between starch and cellulose polymers, most notably in structural configuration, physicochemical properties, and susceptibility to certain enzymes. Because of its  $\beta$  configuration, cellulose forms a sheeted, ribbon-like structure, whereas starch polymers are usually helical. The  $\alpha$  configuration and the helical geometry of starch contribute to its unique properties and enzyme digestibility. Starch polymers can be hydrolyzed by amylase enzymes, often referred to as the “starch-splitting” enzyme be digested by most animals. Glucose polymerization in starch results in two types of polymers, amylose and amylopectin. Amylose is an essentially linear polymer, whereas the amylopectin molecule is much larger and is branched. The structural differences between these two polymers contribute to significant differences in starch properties and functionality

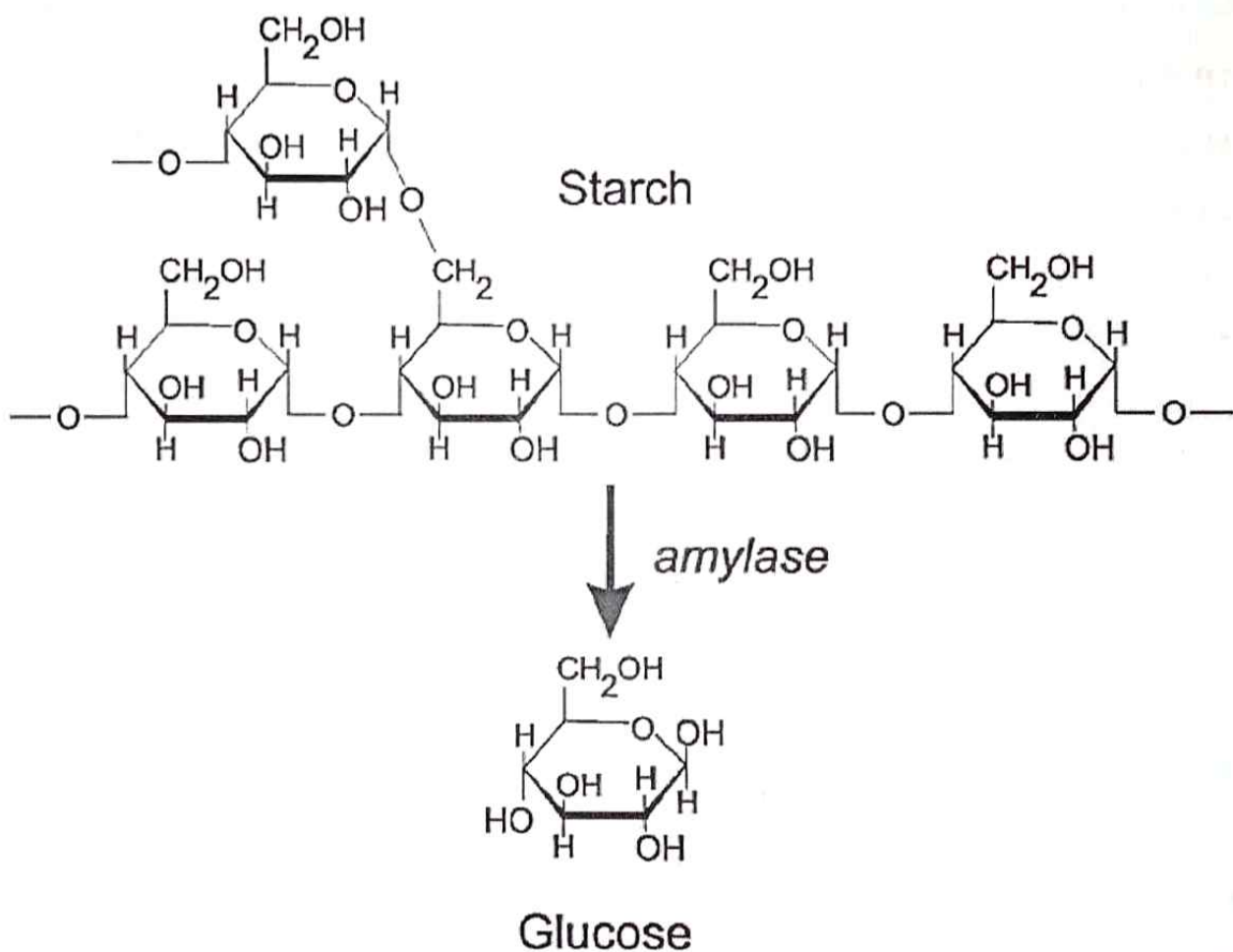
Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of two types of molecules: the linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin by weight. Glycogen, the glucose store of animals, is a more branched version of amylopectin. In industry, starch is converted into sugars, for example by malting, and fermented to produce ethanol in the manufacture of beer, whisky and biofuel. It is processed to produce many of the sugars used in processed foods. Dissolving starch in warm water gives wheatpaste, which can be used as a thickening, stiffening or gluing agent. The biggest industrial

non-food use of starch is as an adhesive in the papermaking process. Starch can be applied to parts of some garments before ironing, to stiffen them.

Enzymes are macromolecules that act as organic catalysts in most of the organism's biochemical reactions that have functions indispensable to maintenance and activity of life. It is crucial to note that reaction rates of certain chemical conversions occurring in living organisms are extremely low, and catalysis is necessary to maintain reasonable time of cell development and division. Majority of functional enzymes are proteins, like trypsin, fumarase or papain. Enzymatic conversion of starch to dextrins and sugars take place by the enzymes obtained from plants, animals, bacteria, and fungi (Fogarty, 1983). Amylase plays an important role in the biogeochemical cycle of carbon and also has a wider application in the biotechnological-based food, detergent, and pharmaceutical industries (Mohapatra et al., 1998).

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into sugar. The structure of amylase, an enzyme that breaks down starches and complex sugars into simple sugars, is usually quite simple, but the enzyme plays an important role in digestion of carbohydrates. There are two different variations of this molecule, called alpha amylase and beta amylase. The human body has two types of alpha amylase, one in saliva and one in the pancreas. Both molecules are important in carbohydrate digestion, since the body cannot digest complex sugars and starches without breaking them down first. Beta amylase is not present in the human body, and is usually found in plants and seeds.

While the structure and type of amylase can vary among organisms, there are some characteristics that all amylase molecules have in common. Alpha and beta amylases both contain a structure called a barrel region, which contributes much of the enzyme's overall shape. The enzymes also all have an essential area called an active site. This is the part of the enzyme that binds to a complex sugar or starch in order to break it down. The process of breaking down carbohydrates in the active site involves interactions between multiple groups of atoms and ions, both in the active site and on the carbohydrate molecule.



**Figure 1- Action of amylase**

## Types of Amylases

### 1. $\alpha$ -Amylase

$\alpha$ -Amylase (E.C.3.2.1.1) is a hydrolase enzyme that catalyses the hydrolysis of internal  $\alpha$ -1, 4-glycosidic linkages in starch to yield products like glucose and maltose. It is a calcium metalloenzyme i.e. it depends on the presence of a metal co factor for its activity . There are 2 types of hydrolases: endo-hydrolase and exo-hydrolase. Endo- hydrolases act on the interior of the substrate molecule, whereas exo-hydrolases act on the terminal non reducing ends. Hence, terminal glucose residues and  $\alpha$ -1, 6-linkages cannot be cleaved by  $\alpha$ -amylase. The substrate that  $\alpha$ -amylase acts upon is starch. Starch is a polysaccharide composed of two types of polymers – amylose and amylopectin. Amylose constitutes 20-25% of the starch molecule. It is a linear chain consisting of repetitive glucose units linked by  $\alpha$ -1, 4-glycosidic linkage. Amylopectin constitutes 75-80% of starch and is characterized by branched chains of glucose units. The linear successive glucose units are linked by  $\alpha$ -1, 4-glycosidic linkage while branching occurs every 15-45 glucose units where  $\alpha$ -1, 6 glycosidic bonds are present.

The hydrolysate composition obtained after hydrolysis of starch is highly dependent on the effect of temperature, the conditions of hydrolysis and the origin of enzyme. The optimum pH for activity is found to be 7.0 . $\alpha$ -Amylase has become an enzyme of crucial importance due to its starch hydrolysis activity and the activities that can be carried out owing to the hydrolysis. One such activity is the production of glucose and fructose syrup from starch.  $\alpha$ -Amylase catalyses the first step in this process. Previously, starch was hydrolyzed into glucose by acid hydrolysis. But this method has drawbacks like the operating conditions are of highly acidic nature and high temperatures.

These limitations are overcome by enzyme hydrolysis of starch to yield high fructose syrup.

The use of enzymes in detergent formulations has also increased dramatically with growing awareness about environment protection. Enzymes are environmentally safe and enhance the detergent's ability to remove tough stains. They are biodegradable and work at milder conditions than chemical catalysts and hence preferred to the latter. There are many such applications of the enzyme which is the driving force behind the research to produce this enzyme in an optimum, safe and convenient manner.



Figure 2. Structure of  $\alpha$ -Amylase

## 2. $\beta$ – Amylase

$\beta$ -Amylase (EC 3.2.1.2) is an exo-hydrolase enzyme that acts from the nonreducing end of a polysaccharide chain by hydrolysis of  $\alpha$ -1, 4-glucan linkages to yield successive maltose units. Since it is unable to cleave branched linkages in branched polysaccharides such as glycogen or amylopectin, the hydrolysis is incomplete and dextrin units remain. Primary sources of  $\beta$ -Amylase are the seeds of higher plants and sweet potatoes. During ripening of fruits,  $\beta$ -Amylase breaks down starch into maltose resulting in the sweetness of ripened fruit. The optimal pH of the enzyme ranges from 4.0 to 5.5.  $\beta$ -Amylase can be used for different applications on the research as well as industrial front. It can be used for structural studies of starch and glycogen molecules produced by various methods. In the industry it is used for fermentation in brewing and distilling industry. Also, it is used to produce high maltose syrups .

## 3. $\gamma$ – Amylase

$\gamma$ -Amylase (EC 3.2.1.3 ) cleaves  $\alpha$ (1-6)glycosidic linkages, in addition to cleaving the last  $\alpha$ (1-4)glycosidic linkages at the nonreducing end of amylose and amylopectin, unlike the other forms of amylase, yielding glucose.  $\gamma$ -amylase is most efficient in acidic environments and has an optimum pH of 3 .

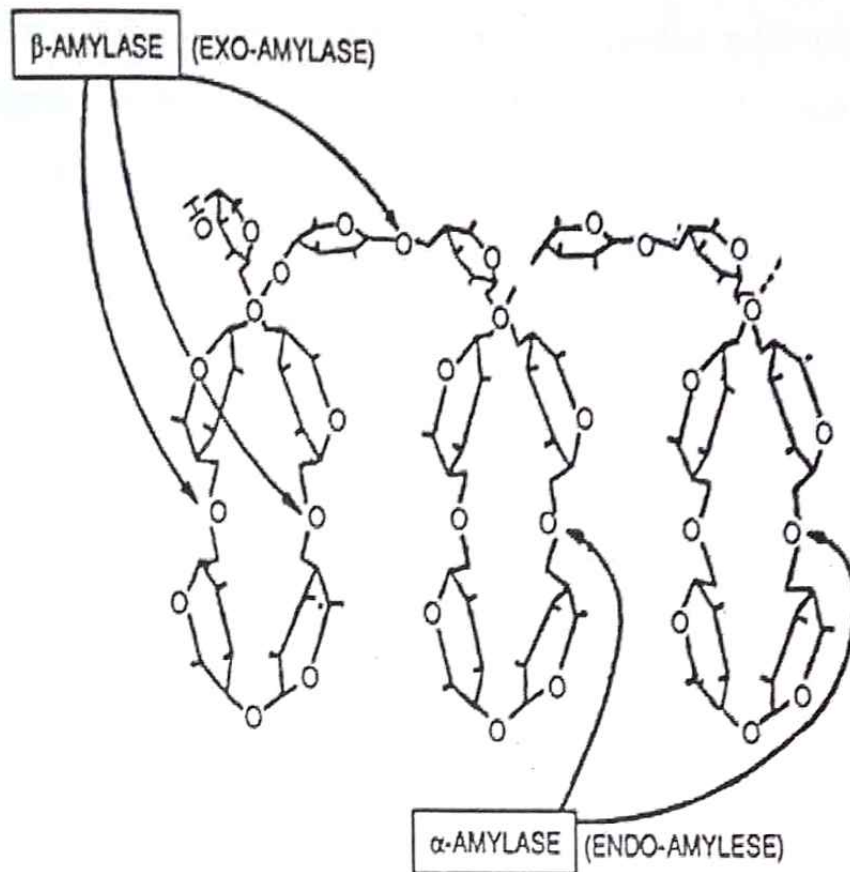


Fig. 4.23. Amylose shown structurally. Notice points where  $\alpha$ - and  $\beta$ -amylases act (modified from Hess, 1975).

Amylases are hydrolyzing enzyme in function which causes hydrolysis of molecules. In biotechnology amylase are of the most important enzymes used[ . They are widely distributed in microbial, plant and animal kingdom. They act by hydrolysing bonds between adjacent glucose units, yielding products characteristic of the particular enzyme involved. Enzymes are the biological catalyst, which initiate and accelerate thousands of biochemical reactions in living cells. Major sources of enzymes are the biological organism plants, animals, and microorganism. Microbial enzymes account the major volume. However, about less than 50 species are actually used to produce the entire microbial enzyme. The potential obviously exists to search for the species producing novel enzymes or enzymes with better properties and yield.

Enzymes produced by the organism are of two types Exoenzymes and Endoenzymes. Exoenzymes which are released from the cell and act on the substrates. These are mainly hydrolytic enzymes that degrade by the addition of

high molecular weight substrates (like polysaccharides, lipid and proteins) into small components (e.g. glucose) that can enter into the cell and are later assimilated. Enzymes required for the hydrolysis of cellulose, starch, pectin, lipid, casein and gelatin belong to the category of exoenzymes. Endoenzymes are utilized by the cell for further metabolic degradation of carbohydrates and are mainly responsible for synthesis. New protoplasmic requirements and production of cellular energy from assimilated materials and these enzymes function inside a cell. Two example of such enzymes are maltose and lactose. Other examples belong to this category include carbohydrate fermentation, nitrate reduction, catalase, urease, IMVic test etc.  $\alpha$ -amylase are exoenzymes that catalysis the hydrolysis of internal  $\alpha$ -1,4-glycosidic linkages in starch in low molecular weight product and such as glucose, maltose, and maltotriose units.

$\alpha$ -amylase is a highly demanded industrial enzymes with extensive commercial applications in various sectors which is carried out with bacterial strain producing extracellular microbial  $\alpha$ -amylase, isolated from soil. Amylase are among the most important enzymes that are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 30% of the world enzymes market. They have opened new frontiers of many commercial biotechnological processes including renewable energy, pharmaceuticals, saccharification or liquefaction of starch, detergents industries, warp sizing of textiles, fibers, paper industries, food staffs, baking, classification of haze formed in beer or fruit juices and for pretreatment of animal feed to improve digestibility .Moreover, microbial amylase have a broad spectrum of industrial applications as they are more stable with great genetic diversity, high enzymatic activity in a wide range of condition and (extreme pH, temperature, osmolarity, pressure etc) simple and cost effective production and easy manipulation to obtain enzymes of desired characteristics.

Today a large number of microbial amylase completely replaced chemical hydrolysis of starch in starch processing industry. The spectrum of amylase applications has expanded in several fields such as clinical, medicinal and analytical chemistry. Starch is an important constituent of the human diet and for this purpose, is used chemically and enzymatically processed into a variety of different product such starch hydrolysates, glucose syrups, fructose maltodextrins derivatives or cyclodextrins used in food industry. Starch contributes greatly to the textural properties of many foods and is widely used in foods and industrial application as thickener, colloidal stabilizer gelling agent and water retention agent . The  $\alpha$ -amylase ( $\alpha$ -1,4 glucan-4-glucanohydrolase) can be found in microorganism, plants and animals.  $\alpha$ -amylase can be produced by different species of microorganism but for commercial applications  $\alpha$ -amylase is derived from the genus Bacillus which is produced from Bacillus licheniformis, B.stearothermophiles and B.amyloliquefaciens. The production of microbial amylase from bacteria is dependent on the type of strain, composition of medium, method of cultivation, cell growth, nutrients requirements, incubation period, pH, temperature, metal ions.

## Sources of amylases

$\alpha$ -Amylase can be isolated from plants, animals or microorganisms. The enzyme has been isolated from barley and rice plants<sup>[7]</sup>. It has been found that cassava mash waste water is a source of  $\alpha$ -Amylase which is active in wide range of pH and temperature. In the recent past, there has been extensive research on microbial production of  $\alpha$ -Amylase. There are 2 major reasons for the increasing interest in microbial sources:

- 1) The growth of microorganisms is rapid and this will in turn speed up the production of enzyme. Microorganisms are easy to handle when compared to

animals and plants. They require lesser space and serve as more cost effective sources.

2) Microorganisms can be easily manipulated using genetic engineering or other means. They can be subjected to strain improvement, mutations and other such changes by which the production of  $\alpha$ -Amylase can be optimized. Also, the microorganisms can be tailored to cater to the needs of growing industries and to obtain enzymes with desired characteristics like thermostability for example. Thermostable  $\alpha$ -Amylases are desired as they minimize contamination risk and reduce reaction time, thus saving considerable amount of energy. Also when hydrolysis is carried out at higher temperatures, the polymerization of D-glucose to iso-maltose is minimized .

$\alpha$ -Amylase is produced by several bacteria, fungi and genetically modified species of microbes. The most widely used source among the bacterial species is the *Bacillus spp.* *B. amyloliquefaciens* and *B. licheniformis* are widely used for commercial production of the enzyme. Other species which have been explored for production of the enzyme include *B. cereus* and *B. subtilis* to name a few.  $\alpha$ -Amylases produced from *Bacillus licheniformis*, *Bacillus stearothermophilus*, and *Bacillus amyloliquefaciens* show promising potential in a number of industrial applications in processes such as food, fermentation, textiles and paper industries *Bacillus subtilis*, *Bacillus stearothermophilus*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens* are known to be good producers of thermostable  $\alpha$ -Amylase.. Enzymes produced by some halophilic microorganisms are stable at high salinities and therefore could be used in many harsh industrial processes where the concentrated salt solutions are used. The halophilic nature the enzyme prevents inhibition of its activity under these conditions which would otherwise occur if a normal enzyme is used. In addition, most halobacterial enzymes are considerably tolerant to high temperatures and remain stable at room temperature over long

periods Halophilic amylases from halophilic bacteria such as *Chromand Bacillus dipsosauri* have been characterized.

Fungal sources of  $\alpha$ -Amylase are confined to terrestrial isolates, mostly to *Aspergillus* species and to only few species of *Penicillium*, *P. brunneum* being one of them<sup>1</sup>. *Penicillium fellutanum* has been used in the recent past to produce  $\alpha$ -Amylase by submerged fermentation. *Penicillium expansum* MT-1 has been used to produce the enzyme by solid state fermentation. Here, Loquat (*Eriobotrya japonica* Lindley) kernels were used as the substrate for growth of the fungi. *Penicillium chrysogenum* was used as the microbial source for producing amylase by solid state fermentation using various substrates such as, corncob leaf, rye straw, wheat straw and wheat bran. The fungal source used predominantly for commercial production of  $\alpha$ -Amylase are the strains of *Aspergillus spp.* *Aspergillus oryzae*, *A. niger* and *A. awamori* are most commonly used species for commercial production among several others.

Genetically modified organisms are also being used for production of  $\alpha$ -Amylase. There are various methods by which microorganisms can be manipulated at a genetic level in order to improve and optimize the production of this enzyme. The microbes can be mutated by chemical agents. Nitrous acids or ethyl methane sulphonate (EMS) have been used for genetic manipulation of bacterial strains *Bacillus amyloliquefaciens* UNG-16 was subjected to mutation by both the chemical (EMS) and radiation method. The mutant strain exhibited an activity of  $102.78 \pm 2.22$  U/ml/min which was 1.4 times greater than the parent strain  $\alpha$ -Amylase production in *B. subtilis* Marburg was improvised by treatment with N-methyl-N'-nitro-N-nitrosoguanidine. One of the mutants, YN9, showed a threefold increase in production of the enzyme when compared to the parent strain. The microbial sources and methods used to produce  $\alpha$ -Amylase are listed in .

## Industrial applications of $\alpha$ -Amylase and the microbial source used

Industrial Application	Microbial source	Role of enzyme	Reference
Starch conversion	<i>B.amyloliquefaciens, B.licheniformis</i>	Hydrolysis of starch into dextrins forming a less viscous starch suspension	[4]
Bakery Industry	<i>B.stearothermophilus</i>	Converting starch in dough to smaller fermentable sugars	[2]
Detergent Industry	<i>B.licheniformis</i>	Digests starch containing foods to water soluble dextrin	[2]
Textile Industry	<i>Bacillus sp</i>	Used in removal of starch sizing agent from woven fabric	[4]
Fuel alcohol Production	<i>E.coli, B.subtilis</i>	Converting starch in to smaller fermentable sugars which are acted upon by yeast to produce alcohol	[4]

The hydrolysis of starch for this purpose was previously carried by acid hydrolysis followed by saccharification using enzymes. This method had many drawbacks. The acidic nature of the process required corrosion resistant material to be used for the equipment and the high temperatures would inactivate the thermolabile enzymes if the hot starch hydrolysate passes into subsequent steps. Hence, enzymatic hydrolysis is a preferred method. The  $\alpha$ -Amylase used in the liquefaction step can be produced from various microbial sources. Thermostable amylases can be employed for hydrolysis at a high temperature. *Bacillus Stearothermophilus*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis* and *Pyrococcus furiosus* are few of the many microbial sources used to produce  $\alpha$ -Amylase that is used in starch conversion

### 1. Bakery Industry

$\alpha$ -Amylase is added to the dough in bread baking process. This causes the starch to hydrolyze into small dextrins which can further be fermented by yeast. This

increases the rate of fermentation. Also the starch hydrolysis decreases the viscosity of the dough, thus improving its texture and increasing loaf volume by rising of dough.

Once the baking is done, there may be changes during storage of baked products. All undesirable changes like increase of crumb firmness, loss of crispness of the crust, decrease in moisture content of the crumb and loss of bread flavor together are called staling. The enzyme is also used as an anti staling agent to improve the shelf life and softness retention of baked goods<sup>1</sup>. Though it has an anti staling effect, a slight overdose may result in gummyness of the bread. This is caused due to production of branched dextrans. In such cases pullulanase is used in combination with amylase resulting in specific hydrolysis of compounds responsible for the gummy nature of amylase treated bread

## **2. Detergent Industry**

The use of enzymes in detergents has increased with the changing methods of dishwashing and laundry. Consumers prefer to use cold water and mild conditions which requires the detergent to work in those imitations. Earlier the chemicals used in detergents caused harm when ingested and the conditions of dishwashing were very harsh. Hence enzymes showed the industry an alternative path. The enzymes are environmentally safe and work at mild conditions.  $\alpha$ -Amylase is used to digest the starch containing food particles into smaller water soluble oligosaccharides. Starch can attract soil particles on to the clothes. Hence removal of starch is also important to maintain the whiteness of clothes. The stability of  $\alpha$ -Amylase at low temperature and alkaline pH contributes to its extensive use in detergents.

The disadvantage of using  $\alpha$ -Amylase is the calcium dependency and oxidant sensitivity of the enzyme. This limitation can be overcome by using  $\alpha$ -Amylase

from genetically modified organisms. Scientists from Novozymes and Genencore International, two major suppliers of detergent enzymes, have worked on this. They replaced the oxidant sensitive amino acid residue *met* at position 197 by *leu* in *B. licheniformis* amylase which resulted in an amylase with improved resistance against oxidative compounds .

### **3. Desizing of Textiles**

Modern production processes in the textile industry can cause breaking of the warp thread. To strengthen the thread, sizing agents are used which strengthen the thread by forming a layer on it and can be removed after the fabric is woven. Starch is a preferred sizing agent as it is easily available, cheaper and can be easily removed from the fabric. The layer of starch is subjected to hydrolysis in the desizing process where  $\alpha$ -Amylase is employed to cleave starch particles randomly into water soluble components that can be removed by washing. The enzyme acts specifically on the starch molecules alone, leaving the fibers unaffected .

### **4. Paper Industry**

Like textiles, paper is also treated with sizing agents to protect it from mechanical strain during processing. The sizing also contributes to better quality of the paper in terms of strength, smoothness, writing and erasability. Starch is commonly used as the sizing agent. The role of  $\alpha$ -Amylase in the paper industry is the partial hydrolysis of starch to make it less viscous in a batch or a continuous process. This is owing to the highly viscous nature of natural starch making it unsuitable for coating on paper .

## 5. Fuel Alcohol Production

Among biofuels, ethanol is most widely used. As starch is an economical starting material, it is used for the production of ethanol as a biofuel. This is done in a series of steps. Firstly, the starch is subjected to liquefaction to form a viscous starch suspension. This is followed by the saccharification process where the starch is hydrolyzed by  $\alpha$ -Amylase to yield fermentable sugars. These sugars are then fermented by yeast to produce alcohol. As an improvisation of this process, protoplast fusion between the amyolytic yeast *Saccharomyces fibuligera* and *S. cerevisiae* was performed to obtain a new yeast strain that can directly produce the biofuel from starch, eliminating the need for a saccharification

### Halophiles

Halophiles are salt-loving organisms that inhabit hypersaline environments. The group includes mainly prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of high salt concentrations. Normally, organisms living in salt-rich environments lose water and die as the result of osmosis. In order to survive in salt-rich environments, the cytoplasm of halophiles must be isotonic with the environment<sup>2</sup>. In order to reach this state, they use two different methods. In the first (mainly used by bacteria, some archaea, yeasts, algae and fungi), organic compounds are stored in the cytoplasm; such compounds help the organism survive osmotic stress<sup>3</sup>.

## MATERIALS AND METHODS

### Materials

- Casuarina fields
- Marine soil samples
- Marine water samples
- Starch agar medium
- Glucose peptone water
- Nutrient agar
- Grams iodine
- Crystal violet
- Safranin
- Malachite green
- Methyl red
- Alcohol
- VP reagent
- Hydrogen peroxide
- Inoculation loop
- Bunsen burner
- Distilled water

## **Glassware**

- Petri plates
- Test tubes
- Conical flasks
- Beaker
- Slides
- Pipettes
- Sterile petri plates

## **Equipment**

- ✓ Autoclave
- ✓ Laminar air flow chamber
- ✓ Hotoven air
- ✓ Incubator
- ✓ Refrigerator

## **composition of starch agar medium**

### **1.Starch Agar Medium**

- ✧ Peptone - 5gm
- ✧ Beef extract - 3gms
- ✧ Nacl - 5 gms
- ✧ Distilled water - 1000ml
- ✧ pH - 7
- ✧ Starch - 10gms

### **2.Nutrient Agar Medium**

- ✧ Peptone - 5gm
- ✧ Beef extract - 3gms
- ✧ Nacl - 5 gms
- ✧ Distilled water - 1000ml
- ✧ pH - 7

## Collection of Marine water Sample



## Collection of Marine soil sample.



## METHOD

Isolation of Amylase Producing Microorganisms Soil samples were collected from different environment sources like soil samples collected from maize fields and marine soil samples collected from vaadarevu beach, chirala. Serial dilution was made and was plated on nutrient agar by spreading 0.1ml of the diluted sample. Then the plates were kept for incubation at 37°C for overnight. Screening for Amylase Activity (Starch Iodine Test) Isolated colonies were picked up from each plate containing pure culture and streaked in straight lines in starch agar plates with starch as the only carbon source.

After incubation at 37°C for 24-48 hrs., individual plates were flooded with Gram's iodine (Gram's iodine- 250 mg iodine crystals added to 2.5gm potassium iodide solution, and 125ml of water, stored at room temperature) to produce a deep blue colored starch-iodine complex. In the zone of degradation no blue colour forms, basis of the detection and screening of an amylolytic strain. The colonies which were showing zone of clearance in starch agar plates were maintained on to nutrient agar slants.

Later the colonies were observed by doing gram staining and spore staining techniques. They were identified as gram positive and spore forming bacteria. later cultures were subjected to biochemical tests like catalase test, MR-VP and citrate utilisation tests were done and identified as some Bacillus sps in both crop soil and marine soil samples.



Bacterial Cultures of  
Crop and Marine soil  
Samples



Starch hydrolysis indicated  
by clear zones after  
addition of Grams iodine

## **Result and Discussion**

The increasing importance of sustainable development has inspired man to use enzymes for various reactions as they are biodegradable and can be produced using biological sources.. The enzyme has crucial applications including the production of fructose syrup, environmentally safe detergents, and baked products. Living in an era of depleting fossil fuels with a desperate need to produce alternate forms of energy, this enzyme is a ray of hope. It is used for biofuel production with starch as a raw material. As the production yields an industrially important enzyme and helps keep the environment clean, more researches are focusing on the microbial production of the enzyme. The present study showed that the isolated amyolytic bacterial strains isolated from both crop soils and marine soils produced appreciable amount of amylase enzyme.

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