Summary
6. SUMMARY

- Amylases are the enzymes which hydrolyze starch (a storage polysaccharide in plants) or glycogen molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose monomer units.

- Amylases are also one of the most important industrial used in starch liquefaction to produce glucose, fructose and maltose and also in brewing, baking, textile, paper, detergent and sugar industries. Amylases have potential application in a number of industrial processes such as in the food, textiles, paper industries, bread making, glucose and fructose syrups, detergents, fuel ethanol from starches, fruit juices, alcoholic beverages, sweeteners, digestive aid and spot remover in dry cleaning. Bacterial α-amylases are now also used in areas of clinical, medicinal, and analytical chemistry.

- The conventional methods of starch hydrolysis using acid has been replaced successfully by processing using starch saccharifying enzymes accounting for approximately 15% share in the world enzyme market.

- The ability to use starch as a carbon and energy source is widely distributed among different organisms. Animals, plants and large variety of bacteria, filamentous fungi and yeast possess starch degrading enzymes to convert it in to usable forms.

- In spite of wide distribution of amylases, microbial sources, namely, fungal and bacterial amylases are useful for industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production, ease of process modification and optimization and can be modified to obtain enzymes of desired characteristics. The major advantage of using microorganisms for the
production capacity is their amino ability, microbial manipulation to obtain the enzyme production of desired character.

➢ Today, the new potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extracellular enzymatic activity in several microorganisms. Microorganisms are the most important sources for enzyme production. Selection of the right organism plays a key role in high yield of desirable enzymes.

➢ Fungi are eukaryotic, achlorophyllous, heterotrophic microorganisms distributed in soil, air, water etc. Fungi are micro-organisms which are well known for their wide range of novelty of enzymes they produce and enzymes of fungal origin are used in the industrial process for which, amount to billions of dollars of revenue annually. Due to their diversity, fungi have been recognized as a source of new enzymes with useful and/or novel characteristics.

➢ Filamentous fungi have a number of properties which make them important both scientifically and industrially. They are widely cultured for the production of amylases on solid state fermentation process owing to their physiological, enzymological and biochemical properties such as their hyphal mode of growth, good tolerance to low water activity.

➢ Western Ghats are regarded as one of the hot spot locations of Biodiversity including biodiversity of microorganisms. Hence the soils from Western Ghats can be potential source amylolytic fungi with respect to various industrial applications.

➢ Solid State Fermentation (SSF) is the growth and/or Cultivation of microorganisms under controlled conditions in the absence of free water for the production of desired products of interest. Solid State Fermentation holds tremendous potentials for the
production of enzymes. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source. This method has economic value for countries with abundance of biomass and agro industrial residues, as these can be used as cheap raw materials.

- Filamentous fungi are the most important group of microorganisms used in SSF process owing to their physiological, enzymological and biochemical properties. The hyphal mode of fungal growth and their good tolerance to low water activity (Aw) and high osmotic pressure conditions make fungi efficient and competitive in natural micro flora for bioconversion of solid substrates. The hyphal mode of growth gives a major advantage to filamentous fungi over unicellular microorganisms in the colonization of solid substrates and for the utilization of available nutrients.

- With the advent of new frontiers in biotechnology, the amylase family enzyme finds potential application in a number of industrial processes.

- Each application of α-amylase requires unique properties with respect to specificity, stability, temperature and pH dependence. Screening of microorganisms with higher α-amylase activities could therefore, facilitate the discovery of novel amylases suitable to new industrial applications.

- Present research work was aimed at the isolation and characterization of fungi from Western Ghat soil, screening them for amylase production, optimization of amylase production by selected isolates, partial purification, study of catalytic properties and characterization of amylases.

- Soil samples were collected from Agumbe region of Western Ghats. Isolation of fungi was done on SDA, PDA, PCA and CZA media by dilution plate method, soil direct plating method, Warcup method and stress techniques. Isolates were
Isolation, Purification and Characterization of Amylases from Potent Fungal Isolates by Solid State Fermentation

preliminarily characterized by macroscopic and microscopic characteristics of colonies. More than 200 isolates were isolated from the soil samples of Western Ghats. The isolates belonged to the genera *Aspergillus* (*A. niger, A. flavus, A. terreus, A. fumegatus, A. nidulans, A. versicolor, A. sydowii*), *Penicillium*, *Trichoderma*, *Fusarium*, *Cladosporium*, *Pacillomyces*, *Gliocladium*, *Scopulariopsis*, *Verticillium*, *Curvularia*, *Alternaria*, *Rhizopus* and *Mucor*.

- The isolates were screened for amylase production in two stages. The primary screening was done by starch agar plate method. 14 isolates which formed maximum hydrolytic zones in primary screening were then selected for the secondary screening. Secondary screening was done on modified CZB media and extracellular protein produced and amylase activities of culture filtrates were estimated by Lowry’s method and DNS methods respectively. After primary screening, 14 isolates *Aspergillus niger, A. terreus, A. flavus, A. oryzae, A. fumegatus, A. versicolor, Aspergillus isolate 16, Aspergillus isolate 25, Aspergillus isolate 43, Aspergillus isolate 199, Penicillium notatum, Penicillium isolate 73, Fusarium sp. and Rhizopus sp.* were selected for secondary screening. Based on the extent of extracellular protein production and amylase activity, two isolates, namely, (*Aspergillus sp. MO 43 and Aspergillus sp. MO 199*) were selected for further studies. The selected isolates were sent to Bioserve Biotechnologies, Hyderabad, for the characterization by ITS sequencing and were identified as *Aspergillus sydowii* and *Aspergillus candidus* respectively.

- The production of amylase by the selected fungal isolates was optimized. The most important parameters such as Solid substrate materials, Carbon sources, Nitrogen sources, pH of the production media, incubation temperature, initial starch
concentration in the production media, effect of incubation period, effect of salinity concentration, effect of different amino acids, inoculum load, effect of light and effect of heavy metals were optimized.

➢ The optimized conditions for amylase production from *Aspergillus sydowii MO 43* are wheat bran as solid substrate material, maltose and the combination of maltose and starch as carbon source, ammonium chloride as nitrogen source, initial pH of the production medium of 6, an incubation temperature of 37°C, an inoculum load of 20% w/v, an incubation period of 120h, additional starch concentration of 1.5%, a salinity concentration of 4%, in presence of light.

➢ The optimized conditions for amylase production from *Aspergillus candidus MO 199* are wheat bran as solid substrate material, glucose and the combination of glucose and starch as carbon source, yeast extract as nitrogen source, initial pH of the production medium of 5, an incubation temperature of 25°C, an inoculum load of 20% w/v, an incubation period of 168h, additional starch concentration of 2%, a salinity concentration of 6%, incubation the in presence of light.

➢ The effect of some important factors such as temperature, pH, incubation period, enzyme concentration, substrate concentration, thermostability and pH stability of the amylases were evaluated.

➢ The study of catalytic properties of amylases of *A. sydowii MO 43* showed that the optimum temperature, optimum pH and optimum incubation period to be 40°C, pH 8 and 50 min. respectively. The enzyme was found to be stable at 40°C and for wide pH ranges from 6 to 10. The optimum enzyme concentration and substrate concentration were noticed to be 0.5 ml and 4 mg respectively.
The study of catalytic properties of amylases of *A. candidus* MO 199 showed that the optimum temperature, optimum pH and optimum incubation period to be 50°C, pH 5 and pH 6 and 50 min. respectively. The enzyme was found to be stable at 50°C and for wide pH ranges from 5 to 10. The optimum enzyme concentration and substrate concentration were noticed to be 0.5 ml and 3 mg starch respectively.

The fungal amylases were subjected for partial purification by ammonium sulphate fractionation, dialysis and ion exchange chromatography. The active fractions from ion exchange chromatography were subjected to SDS PAGE for the estimation of approximate molecular weight of the amylases. The bands of amylases in the electrophoretic gel were sent to Chromous lab, Bangalore for protein sequencing by LC-MS/MS.

The approximate molecular weight of the amylases of *A. sydowii* MO 43 and *A. candidus* MO 199 determined by SDS-PAGE were found to be 40 kDa and 50 kDa respectively.