

## STUDY ON EXTRACELLULAR AMYLASE FROM HALOPHILIC BACTERIUM

Aadil Khan

Institute of Biological sciences

Sarhad University of Science and Information Technology, Peshawar.

### Abstract

Halophiles are organisms that live in hypersaline environment. They may be fungal, bacterial or archaeal species. Halophiles are also known for the production of important enzymes that are involved in many industrial process due to stability in harsh conditions e.g, stability at acidic pH, high temperature and high NaCl concentration. One of the most important enzyme is amylase that is involved in many industries such as starch, detergent and textile. In this study, four halophilic strains (S6, S8, S12 and S16) were isolated from the Bahadur Khel salt mines in Karak, Pakistan. The isolated strains were screened for amylase activity at different NaCl concentration on nutrient agar plates containing 0.5% starch as a substrate and incubated at 37°C for 48hrs. Out of four strains S8 was found amylase positive by showing better activity at 8% NaCl, confirming the strain as moderate halophile. The amylase activity was surprisingly found at pH values of 2, 3, 5, 6, 7 and 8. Protein were precipitated in a 72hrs old culture of strain (S8) using ammonium sulfate precipitation and reconstituted in Tris buffer (pH 7). Crude amylase stable at temperature range 37-55°C after incubation for 30 minutes. Crude amylase produced a zone of 20mm on starch agar plates. DNA was extracted and run on gel from the amylase positive strain. 16S rRNA gene sequences revealed that the halophilic strain (S8) *Halomonas elongate* by showing 99% similarity. This study concludes that various halophilic species are present in Karak salt mines that can produce numerous enzymes, one of them is amylase, which can help in improving life.

### Introduction:

Halophilic microorganisms require extremely high salt (2 to 5 M NaCl) focuses for development and are found in salterns and hypersaline lakes. Numerous outrageous and moderate halophiles have been disengaged and explored for conceivable biotechnological applications. These incorporate the creation of  $\beta$ -carotene, polyhydroxy alkanooates, proteins and good solutes, upgraded oil recuperation and debasement of poisonous synthetic substances that can contaminate hypersaline natural surroundings. Furthermore, halophiles produce exozymes, for example, amylases, proteases and nucleases of expected business esteems. Halophilic proteins are recognized from their homologous proteins by showing momentous unsteadiness in arrangements with low salt focuses and by keeping up solvent and dynamic adaptations in high groupings of salt upto 5 M NaCl. There are number of compounds of this sort created by

some halophilic microorganisms that have ideal movement at high salinities and could in this way utilized in numerous unforgiving modern procedures where the concentrated salt arrangements utilized would somehow or another hinder numerous enzymatic changes.

Halophilic microorganisms assume a fundamental job in different aging procedures that happen within the sight of salt. The high salt resistance of extraordinary halophiles empowers their development under non-sterile and therefore cost-decreasing conditions. Up until now, a few wellknown proteases, for example, bromelain, papain, and pepsin have been utilized as biocatalysts of protein hydrolysis in fish sauce aging. Be that as it may, a large portion of these proteases were not adequately stable within the sight of high salt fixation. Halophilic living beings have developed in saline condition and can defeat the deteriorous impacts of salt, and their proteins require salt, holding movement in salt upto immersing fixation. Some halophilic proteases have been sanitized, described, indicated most extreme action at impartial pH at a temperature running from 55-66°C. Halophilic proteases have wide application in the handling of food, cowhide and cleansers. Given the likely utilizations of halophilic proteases and the need to scan for new halophilic living beings creating most extreme catalyst creation is a ceaseless procedure. We have announced the ideal culture conditions for the creation of halophilic protease from *Chromohalobacter* sp. TVSP101. In the current examination we report the purging and portrayal of a very halophilic, thermophilic protease from a recently detached *Chromohalobacter* sp. TVSP101.

Alpha amylase, arbitrarily assault  $\alpha$ -1, 4-glycosidic obligation of starch, maltodextrins, maltose, and glucose units were shaped because of  $\alpha$ -amylase activity (Maity et al. 2015). Alpha amylase has increased an extraordinary consideration because of its expansive range of uses and financial benefits (Bansode 2010). Right now, amylase creation has reached up to 65% of chemical market on the planet and is constantly expanding (Abdullah et al. 2014). It has a wide scope of uses in starch liquefaction, paper, desizing of material textures, getting ready starch coatings of paints, evacuating backdrop, blending industry, sugar enlistment by the creation of sugar syrups, and pharmaceuticals. Alpha amylase can be delivered by miniaturized scale and macroorganisms (Simair et al. 2017). At the point when the starting points are in correlation, amylases from creature and plant roots have low obstruction under

acidic, fundamental, and high temperature conditions. Then again, bacterial and contagious amylases have great strength under such conditions and have progressively financial creation activities. In this way, microbial proteins have been applied in an enormous number of utilizations.

As of late, starch saccharification, the primary utilization of amylase, has completely uprooted substance usage with amylase chemical hydrolysis. Saccharification is executed at raised temperature and thermophilic microorganisms could be most cheerful contender for amylase creation in light of the fact that these strains will deliver thermostable amylase. This is the reason, despite everything, look for new microbial strains is kept on accomplishing modern prerequisites of proteins.

Also, amylase is enhanced in nearby cleansers because of high basic pH dependability required for ventures (Asad et al. 2011). In addition, thermophilic amylase is required for different applications in the creation of sugars from starch and saccharification of starch for biochemical creation (Castro et al. 1999).

Bacillus class is celebrated for alpha-amylase creation and a few Bacillus strains, for example, *B. stearothermophilus*, *B. subtilis*, *B. cereus*, *B. licheniformis*, and *B. amyloliquefaciens* are separated and screened for amylase creation (Sivaramakrishnan et al. 2006). Some Bacillus strains are associated with crude starch debasements (Demirkan et al. 2005; Goyal et al. 2005; Puspasari et al. 2013).

All things considered, many are being looked to get the most extreme yield with exceptional modern properties. Protein creation could be improved through the advancement of different physical and dietary development boundaries; the significant components that decide the bioprocess are brooding period, temperature, pH, air circulation, inoculum size, carbon, and nitrogen sources. Accordingly, in this examination, the impacts of physical and dietary boundaries were researched for the ideal creation of extracellular protein  $\alpha$ -amylase from *Bacillus* sp. NRC22017.

Keywords: HALOPHILIC BACTERIUM, bacterial and contagious amylases, Bacillus strains