

## ABSTRACT

Amylase is an enzyme that can break down starch into its monomers i.e., dextrin and maltose. This enzyme has versatile applications in bioethanol production, as a detergent additive, food and beverage, textile, and pharmaceutical industries. This research mainly aims to isolate new strains and check their amylolytic activity, optimization, and purification of the amylase enzyme. The isolated strain was identified by 16S rRNA sequencing as *Brevibacillus thermoruber*. Different parameters such as media, temperature, pH, time of incubation, carbon and nitrogen sources, inoculum size, and metal ions were optimized for maximum amylase production. *B. subtilis* and *Br. thermoruber* showed the optimum amylase production after 48 hours of incubation, 4% inoculum size, maltose as a carbon source, and yeast extract as a nitrogen source at 37 °C and 55 °C respectively. *B. subtilis* grew best at pH 6 while *Br. thermoruber* grew both at acidic (pH 6) and alkaline conditions (pH 10). The optimum enzyme concentration and activity achieved were about 2.682 µg/ml and 178.82 U/ml respectively from *B. subtilis* while 2.366 µg/ml 157.77 U/ml correspondingly from *Br. thermoruber*. Different types of metal ions were also used for optimization, and it was observed that BaCl<sub>2</sub> and CuSO<sub>4</sub> showed a slight effect, but MgSO<sub>4</sub> has almost no effect on the enzyme activity of *B. subtilis*. However, BaCl<sub>2</sub>, CuSO<sub>4</sub>, and MgSO<sub>4</sub> have a strong effect on enzyme activity of *Br. thermoruber*. NiCl<sub>2</sub> has strong effects on the activity of both strains while CaCl<sub>2</sub> has a slight effect on the enzyme activity of *Br. thermoruber* as compared to other metal ions. Ammonium sulfate precipitation (10-70 %) was performed for the purification of amylase enzyme it was observed that *B. subtilis* enzyme precipitated out at 60% and its specific activity is about 216.48 U/mg/min and purification fold was 4.86 while *Br. thermoruber* precipitate out at 40% and its specific activity was about 176.235 U/mg/min and purification fold is 3.36. SDS PAGE was used to determine the molecular weight of the amylase enzyme which was about 72 kDa and 56 kDa of *B. subtilis* and *Br. thermoruber*. Amylase enzyme compatibility was checked with the commercial detergent, and it was concluded that heated detergent could not remove the ketchup stain from the fabrics but when it was mixed with amylase enzyme it showed much better results. Amylase enzyme can be used for bioethanol production, rice husk was a much better source as compared to potato peel and a much higher quantity of bioethanol is produced by using rice husk.