ABSTRACT

Soil microbiota plays a critical role in sourcing microbial enzymes such as α-amylases, which hold a substantial share of the global enzyme market. This study involved collecting 20 soil samples and plant materials from different areas. From these samples, 50 bacterial strains were isolated, out of which 15 exhibited starch hydrolysis activity, with two promising strains selected for fermentation optimization. Various parameters, including medium type, temperature, pH, incubation period, agro-waste substrates, and organic and inorganic nutrient sources, were optimized. After 16S rRNA sequencing, the strains were identified as *Brevibacillus reuszeri* and *Brevibacillus brevis*. The optimal conditions for maximizing amylase production were determined to be 37°C, pH 7, and a 72-hour incubation period. Among the agro-waste substrates combined with starch, wheat bran proved the most effective for *Brevibacillus brevis*, while potato peel showed the highest efficacy for *Brevibacillus reuszeri*. Medium optimization by Response Surface Methodology (RSM) increased amylase production, with *Brevibacillus reuszeri* reaching 290 µmol/ml and *Brevibacillus brevis* achieving a maximum activity of 425 µmol/ml. A comprehensive enzyme purification protocol was optimized, resulting in 17-18 fold increase in specific activity.