

ABSTRACT

α -Amylases being the most protean, primeval and widely used starch degrading enzyme covers approx. 30% of total enzyme market shares. It randomly hydrolyze α -(1,4)-D-glucosidic linkage of starch into progressively small and diverse products, releasing byproducts such dextrins and polymers of glucose units. It has many paramount applications including paper, detergent, pharmaceutical, textile, drug delivery, biofilm inhibitor, bioremediating agent, starch processing and biodemulsifier. Ribotyping, a specific, rapid most accurate method in molecular technique that is used for strain identification. In this technique, 16S rRNA for prokaryotes is analyzed for identification of unknown strain. The strain was isolated from local yogurt sample, Pakistan. Genomic DNA isolated from strain was subjected to polymerase chain reaction using 16S rRNA gene forward and reverse universal primers. The size of amplified 16S rRNA gene was 1.5 KB and was purified from the gel using Gene JET gel extraction kit (Fermentas, Cat# K0691). The gene clean and stock of isolated strain was sent to Humanizing Genomics Macrogen. Sequencing results showed that the strain was *Limosilactobacillus fermentum*. Different enzymatic assays were performed and strain showed ability to produce α -amylase, lipase and xylose isomerase. Production optimization of α -amylase showed that *Limosilactobacillus fermentum* was able to produce maximum enzyme units (60.74 mg/min/ml) when grown on MRS medium of pH 8.0 at 37 °C for 24h having 2% inoculum volume (v/v), 1% starch concentration (w/v), 0.5mM Ca²⁺, 1% potato peels, 1% amylose, 1% yeast extract and 1% ammonium citrate. SDS-PAGE showed the molecular weight of α -amylase protein was ~100 kDA.