

## Preliminary Studies on the Isolation and Selection of Bacterial Strains to Produce Thermo Active $\alpha$ -Amylase

Vaseekaran. S, Balakumar. S and Arasaratnam. V  
*Department of Biochemistry, University of Jaffna, Sri Lanka.*

The present study is aimed at isolating thermostable  $\alpha$ -amylase producing bacterial strains. For this purpose 72 bacterial strains capable of hydrolyzing starch at pH 7.0 [in medium containing (g L<sup>-1</sup>) nutrient agar, 25.0; agar, 10.0; and soluble starch, 3.0] were isolated from different sources such as soil (42), gruel of rice (09), kitchen waste (06), bakery waste (08), flour mill waste (04) and tea waste (03) by incubating at 37°C for 24h. Single colonies with different sizes [2-4mm (48) and 5-7mm (24)], shapes [round (55), irregular (12) and filamentous (5)], elevations [flat (42) and low convex (30)], colours [pale (34), white (29) and yellow (9)] and margins [entire (55) and irregular (17)] were selected.  $\alpha$ -Amylase producing strains were selected based on halo formation with Gram's iodine on starch-nutrient agar plate. Strains which produced clear halos were grown on starch-nutrient agar slants, transferred in to activation medium and incubated in a shaker water bath at 42°C and 120 rpm for 12h. The activated strains were transferred to fermentation medium under the same conditions. Among the 72 strains, 5 strains showed the highest colony diameter to halo diameter ratio. They also produced the highest  $\alpha$ -amylase activities in the range of 1-7 U mL<sup>-1</sup> at 24h. Among these 5 strains, 3 strains were selected based on their  $\alpha$ -amylase production and named as S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>. When the three strains were grown in fermentation medium at 80°C and at pH 7.0  $\alpha$ -amylase activities produced by S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> at 24h were 7.0(±0.21), 5.52(±0.31) and 4.73(±0.27) U mL<sup>-1</sup> respectively. The optimum temperature of the fermentation to produce highest  $\alpha$ -amylase activity was 42°C for all three strains. When the effect of fermentation period on  $\alpha$ -amylase production was studied all three strains produced highest  $\alpha$ -amylase activities at 24h. Even though the strains S<sub>2</sub> and S<sub>3</sub> produce enzymes with similar kinetic properties they show differences in colony morphology. [The colony morphology (shape, elevation, margin and colour) of the strains S<sub>2</sub> and S<sub>3</sub> were circular, low convex, entire and pale and circular, flat, irregular and pale respectively.] Therefore the strains S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> were selected for further studies.

**Key words:**  $\alpha$ -Amylase, Thermostable, strains and halo.