Isolation of α-amylase producing bacteria by Gel Micro Droplet method

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When conventional microbial cultivation conditions are used to study populations present in environmental samples, almost 99% of the microbial diversity is unable to grow and form visible colonies on agar-plates. These unrecovered microorganisms represent the source for novel enzymes or other metabolites, which could be of biotechnological interest. The aim of this study was to isolate an α-amylase producing bacterium. Gel Micro Droplets (GMDs) were formed by quickly spraying the polymer solution (diluted cell suspension containing sample, 5ml mixed with 50ml of sterile, pre-reduced 3%(w/v) sodium alginate containing 0.45% soluble starch) through a needle with 0.2mm diameter into 0.5M $CaCl_2$ solution to form micro dropletets or micro beads to contain approximately one cell per bead. Isolated bacteria were cultured in enrichment medium containing (gl^{-1}) starch 5.0; yeast extract 1.0; and NaCl 1.0 and transferred to nutrient agar media containing 0.3% starch. Ninety samples were isolated from horse dung (60 samples) and lake soil samples (30 samples) using the GMDs method. Isolated 60 strains were transferred from slants to activation medium containing (gl^{-1}) meat extract 10.0; peptone 10.0; NaCl 3.0 and soluble starch 3.0 at pH 7.0. Among these strains, 22 strains showed growth (OD) more than 1.00 at 610 nm. Of the 22 strains 7 were from horse dung and 15 were from lake soil samples. These 22 strains were activated in activation medium at 37°C and at 120 rpm for 16h and transferred (20% v/v) to fermentation medium containing (gl^{-1}) soluble starch 3.0; $CaCl_2.2H_2O$ 0.005; $MgCl_2.6H_2O$ 0.005; $FeCl_3$ 0.005; K_2HPO_4 2.5; KH_2PO_4 1.0; peptone 2.0; NaCl 1.0; and $(NH_4)_2SO_4$ 2.0 at pH 7.0 and incubated at 37°C. All the seven strains from horse dung utilized starch but did not produce α-amylase. Among the fifteen bacterial strains selected from lake soil samples, all the strains utilized starch, two strains did not produce α-amylase while nine strains produced α-amylase activity between 0-0.5Uml^{-1}, one strain produced α-amylase activity between 0.51-1.0Uml^{-1}, two strains produced α-amylase activity between 1.0 and 1.5Uml^{-1}, and one strain produced α-amylase activity between 3.5 and 4.0Uml^{-1} at 24h. Among the strains isolated from lake soil, strain SL19 showed highest α–amylase activity (5.88 Uml^{-1}) at 48h in fermentation medium and hence selected for further studies. The strain SL19 gave highest α-amylase activity in the activation medium (6.8 Uml^{-1}) than in the fermentation medium (4.84 Uml^{-1}) at 36h. The α-amylase produced by strain SL19 was active at pH 7.0 and from 55 to 95°C, showing the highest activity at 70°C. SL19 produced α-amylase was active at 70°C and from pH 3.0 to 9.0 showing highest activity at pH 7.0 and 95% of highest activity at pH 9.0.The enzyme showed 100% stability for 1hour at 70°C and pH 7.0. Further studies are in progress to characterize the strain.

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