Optimization of Media Compositions to Improve α-Amylase Production by *Bacillus licheniformis* ATCC 6346

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Thermostable α-amylase is produced by a mesophilic organism *Bacillus licheniformism.* Single colony of *Bacillus licheniformis* ATCC 6346 from nutrient slants (grown at 37°C for 24h) was transferred to activation medium and incubated at 42°C in a rotary shaker (100rpm) for 12h and used as inoculum. The fermentation medium was inoculated with inoculum (20%, v/v) and incubated at 42°C and 100rpm. The fermentation medium contained (gL^{-1}) soluble starch, 2.0; $(NH_4)_2SO_4$, 1.0; peptone, 2.0; NaCl, 1.0; $FeCl_3$, 0.005; $MgCl_2.6H_2O$, 0.005; $CaCl_2.2H_2O$, 0.005; KH_2PO_4, 1.0 and K_2HPO_4, 2.5. Production of α-amylase in the fermentation medium was 20.1 UmL^{-1}. Above compositions of fermentation medium were optimized to improve the α-amylase production by *B.licheniformis* ATCC 6346. The soluble starch concentration in the medium was varied in the range of 2-10gL^{-1} while all other contents of the fermentation medium were kept the same. The highest α-amylase activity (28.7UmL^{-1}) was produced in the fermentation medium containing 4gL^{-1} soluble starch. Then to the fermentation medium containing 4gL^{-1} soluble starch, different concentration of $(NH_4)_2SO_4$ (2-9gL^{-1}) was added and the highest enzyme activity (39.6UmL^{-1}) was obtained in the medium containing 5gL^{-1} $(NH_4)_2SO_4$. In the presence of 4gL^{-1} soluble starch and 5gL^{-1} $(NH_4)_2SO_4$, when the concentration of K_2HPO_4 in the medium was varied from 0.5 to 9.5 gL^{-1}, maximum (40.8UmL^{-1}) enzyme activity was obtained in the presence of 7.5gL^{-1} K_2HPO_4 at 48h. In the presence of optimized amount of soluble starch, $(NH_4)_2SO_4$ and K_2HPO_4, when the amount of KH_2PO_4 in the media was varied from 0.5 to 9.0gL^{-1}, the α-amylase produced in the medium containing 4.0gL^{-1} KH_2PO_4 was the highest (41.4UmL^{-1}). Then to the fermentation medium containing 4gL^{-1} soluble starch, 5gL^{-1} $(NH_4)_2SO_4$, 7.5gL^{-1} K_2HPO_4 and 4.0gL^{-1} KH_2PO_4, the peptone concentration in the medium was varied in the range of 1-12gL^{-1}. The highest α-amylase activity (43.2UmL^{-1}) was produced in the fermentation medium containing 6gL^{-1} peptone. Then to the fermentation medium containing optimized amount of soluble starch, $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4 and peptone, different concentrations of NaCl (0-4gL^{-1}) was added and the highest α-amylase activity (44UmL^{-1}) was obtained in the absence of NaCl at 48h. In presence of 4gL^{-1} soluble starch, 5gL^{-1} $(NH_4)_2SO_4$, 7.5gL^{-1} K_2HPO_4, 4.0gL^{-1} KH_2PO_4 and 6gL^{-1} peptone, when the amount of

$CaCl_2.6H_2O$ in the medium was varied from 0.005 to 0.045gL^{-1}, α-amylase production in the medium which had 0.01gL^{-1} $CaCl_2.2H_2O$ gave the highest α-amylase activity (44UmL^{-1}) at 48h, at 42°C and 100rpm. Then to the fermentation medium containing optimized amount of soluble starch, $(NH_4)_2SO_4$, K_2HPO_4 , KH_2PO_4, peptone and $CaCl_2.2H_2O$, when the amount of $MgCl_2.6H_2O$ in the medium was varied from 0.005 to 0.045gL^{-1}, highest α-amylase activity (45UmL^{-1}) was produced at 48h in the media containing 0.01gL^{-1} $MgCl_2.6H_2O$. In presence of optimized amount of soluble starch, $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4, peptone, $CaCl_2.6H_2O$ and $MgCl_2.6H_2O$, when the amount of $FeCl_3$ in the medium was varied from 0.005 to 0.045gL^{-1}, highest α-amylase activity (44.1UmL^{-1}) was obtained at 48h, in the medium containing 0.01gL^{-1} $FeCl_3$ when compared to control, which contains 0.005gL^{-1} $FeCl_3$ (43UmL^{-1}). Thus optimizing the concentration of the components to 4gL^{-1} soluble starch, 5gL^{-1} $(NH_4)_2SO_4$, 7.5gL^{-1} K_2HPO_4, 4.0gL^{-1} KH_2PO_4, 6gL^{-1} peptone, 0.01gL^{-1} $CaCl_2.6H_2O$, 0.01gL^{-1} $MgCl_2.6H_2O$ and 0.01gL^{-1} $FeCl_3$ have improved the α-amylase production from 20.1 to 44.1UmL^{-1}.