The Chitosanase - The Study of Enzyme Fermentation Condition and Enzyme Characterization from Sinorhizobium sp

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ABSTRACT

A bacterial strain capable of utilizing chitosan as the sole carbon source was isolated from soil and was identified as a member of the genus Sinorhizobium sp. This strain, designed CSJ01, produced extracellular chitosan-degrading enzyme in the presence of chitosan. In this study, the fermentation condition and characterization of the chitosanase were investigated. This crude chitosanase was stable in the alkaline side. Optimum pH was about 6, and stable pH in the incubation at 40°C for 30 min was 3-10. Optimum temperature was around 40°C, and enzyme activity was relatively stable in the range of 20-40°C. The presence of sodium and iron ions promoted the activities; however, the enzyme activity was inhibited by calcium ion. The partial purified chitosanase was obtained by salting out with ammonium sulfate and by adsorbing with colloidal chitosan. The molecular weight was estimated to 25, 43, 50 or 63 kDa on SDS-PAGE. This chitosanase hydrolyzed colloidal 82% N-deacetylated chitosan to chitobiose and chitotetraose. The production of Sinorhizobium sp. CSJ01 chitosanase was affected by temperature, initial pH, rotational speed, and medium composition, such as the concentrations of colloidal chitosan, yeast extract, KH2PO4, MgSO4.7H2O, MnSO4. In addition, the enzyme production was affected significantly by the initial pH of medium and the concentrations of chitosan and yeast extract. In this study, the optimal cultivation condition was investigated using response surface methodology (the factors were initial pH, the concentrations of colloidal chitosan and yeast extract). The Experimental results from 23 factorial and central composite design showed the optimal density of factors were colloidal chitosan 0.8%, yeast extract 0.4% and initial pH 9.1. The optimal condition for the production of Sinorhizobium sp. CSJ01 chitosanase was colloidal chitosan 0.8%, yeast extract 0.4%, KH2PO4 0.1%, MgSO4.7H2O 0.05%, MnSO4 0.001%, temperature 30°C, initial pH 9.1, rotational speed 200 rpm.

Keywords : Sinorhizobium sp. ; chitosan ; chitosanase ; response surface methodology

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