Abstract

Heparosan is a commercially expensive glycosaminoglycan (GAG), composed of repeating disaccharide units of GlcA and GlcNAc linked by α (1→4) and β (1→4) glycosidic bonds. Heparosan production through microbial fermentation is a promising method to prepare chemoenzymatic heparin. Until now, *E. coli* K5, *P. multocida* type D and genetically modified microorganisms have been used to produce heparosan. *Bacillus megaterium*, a safe gram-positive bacterium has been an important industrial host due to its superior characteristics such as the efficient expression of heterologous genes, stable plasmid maintenance, lack of alkaline protease activity and efficient se-cretion capability. The first chapter of the thesis deals with engineering a functional heparosan synthesis pathway in *Bacillus megaterium* by the expression of *E. coli* K5 *kfiC* and *kfiA* glycosyl-transferase genes. Upregulation of individual UDP-sugar precursor pathway genes enhanced the heparosan production, indicating that UDP-precursor sugar concentrations were limiting the biosynthesis. The engineered *B. megaterium* yielded a maximum heparosan concentration of 394 mg/L in batch biore-actor. The heparosan titer was further increased to 1.32 g/L in fed-batch fermentation. The heparosan molecular weight varied from 31 to 60 kDa, indicating its potential as a precursor for chemoenzymatic heparin synthesis. The second chapter of the thesis deals with the development of dual promoter expression system for heparosan production in *B. megaterium*. In the previous chapter, *kfiC* and *kfiA* genes were expressed in polycistronic manner, resembling the *E. coli* K5 gene cluster. We observed an unbalanced expression of KfiC and KfiA proteins. Hence, dual promoter plasmid system was constructed to increase the expression levels of KfiC and KfiA proteins. Dual promoter plasmid system along with UDP-glucuronic acid pathway overexpression (CADuet-DB) increased the heparosan production to 203 mg/L in shake flask experiments. Batch and fed-batch fermentation of strain CADuet-DB under controlled conditions yielded a maximum heparosan concentration of 627 mg/L and 1.96g/L, respectively. The third chapter deals with the influence of sucrose and GlcNAc concentration on Biomass growth and heparosan production. The heparosan production significantly in- fluenced by certain factors like substrate concentration and process conditions. Thus, determining the substrate concentration to identify the inhibition phenomenon on hep- arosan production and *B. megaterium* growth. Further, experimentaldata was modeled using modified logistic equation and the kinetic parameters were determined. we studied the effect of precursors (N-acteylglucosamine and glucuronic acid) concentration on heparosan production. Batch fermentation with sucrose and N-acteylglucosamine resulted in the highest heparosan concentration of 911 mg/L in batch fermentation under optimal conditions. We found the simultaneous consumption of sucrose and N-acetylglucosamine, hence interactive multi substrate kinetic model was used to describe the biomass growth and heparosan production. Overall, this thesis addressed a safe approach to synthesize heparosan from *B. megaterium*, which have potential applications as a heparin precursor and in drug delivery applications.