P33-17

Geobacillus sp. 95 as a source of enzymes with special characteristics: Characterization of thermostable lipases, esterases, ureases and nitrate reductases

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Geobacillus bacteria are widely distributed and readily isolated from natural and man-made thermophilic biotopes. As with many thermophiles, considerable interest in potential industrial application of these bacteria and their thermostable enzymes exists. In previous works we have shown that *Geobacillus* sp. 95 strain produced lipolytic enzymes (GD-95 lipase and GDEst-95 esterase) with attractive characteristics for industrial application. It has been shown that a chimeric variant composed of these two enzymes also demonstrated high potential for the lipid bio-industry.

In this work we analysed the potential of several *Geobacillus* spp. strains to produce ureases, which can be applied in biocement production and microbial calcium carbonate precipitation. Screening by classical microbial methods using selective urea-broth medium and molecular detection by α subunit-specific primers allowed the selection of *Geobacillus* sp. 95 as a perspective urease-producing strain. Another important industrial and pharmaceutical application of microorganisms is production of silver nanoparticles. The most significant enzyme in bacterial-based silver nanoparticle synthesis is nitrate reductase. In this study it was shown that *Geobacillus* sp. 95 strain could successfully perform synthesis of silver nanoparticles and possessed high nitrate reductase activity. Based on the obtained experimental results, *Geobacillus* sp. 95 strain and thermostable enzymes produced by this strain show high perspectives for further application in industry.

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Recombinant protein production and purification

P34-1

Optimizing cell density of *Pichia pastoris* for production of recombinant hepatitis B surface antigen via employing short-period continuous operation

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In biopharmaceutical industry, high cell density fed-batch cultivation is the main approach for production of recombinant protein with high yields in methylotrophic yeast *Pichia pastoris* (*P. pastoris*). In current study, in addition to conventional fed-batch process, we employed short-period chemostat fermentation to control and manipulate the cell density of *P. pastoris* in the pilot-scale fermenter easily. For this purpose, after reaching the maximum broth volume of 5 L in the fed-batch process, the operation mode was changed to chemostat fermentation, under the DO-stat condition, with the dilution rate of 0.009, and the process continued until reaching the steady-state point. To alter the cell density in the chemostat fermentation stage, the inflow of methanol, as a limiting nutrient, was adjusted to various values between 31 ml/h and 42 ml/h. In each selected point, the cell density, methanol consumption rate and amount of both total protein and recombinant hepatitis B surface antigen (rHBsAg) were measured. According to the results, the optimal methanol inflow based on yield, productivity and ease of process control was 40 ml/h. In this flow rate the cell density increased from 363 mg/ml WCW in the fed-batch stage to 450 mg/ml WCW. In the selected operation condition, the titer, volumetric and specific productivity were 188.8 mg/l, 1.7 mg/l/h and 0.00468 mg/g/h, respectively. The obtained cell density is suitable for large-scale fermentation process without creating any major issues such as excessive heat dissipation or very low concentration of dissolved oxygen.

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P34-2

Affinity purification of recombinant proteins using a LysM domain and bacterium like particles

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The lysin motif (LysM) is a ubiquitous motif across kingdoms, which in bacteria allows cell wall degrading enzymes to bind non-covalently to peptidoglycan. This property has been exploited for two decades to design mucosal vaccines consisting of LysM-tagged recombinant proteins anchored to bacterium like particles (BLP) as carriers. Surprisingly, less attention has been paid to apply the LysM motif to protein purification of recombinant proteins. Thus, our goal was to determine if the LysM motif is suitable for recombinant protein purification.

We obtained the BLPs by treating overnight cultures of lactobacilli with acid and heat to get rid of other cell wall components that may interfere with binding. To select the best binding matrix, we generated BLPs from 3 different *Lactobacillus* species: *L. rhamnosus, L. fermentum*, and *L. vaginalis* and checked them by transmission electron microscopy. We constructed a fusion protein consisting of the yellow fluorescent protein Venus fused to a module containing five LysM motifs derived from a *Lactobacillus* sp. strain. The recombinant protein was expressed in *E. coli* Rossetta using standard procedures, and the supernatant containing the fusion protein was incubated with BLPs for binding. We evaluated the effectiveness of binding by fluorescent microscopy and SDS-PAGE. After binding, the complex was washed several times, and the elution of the protein was tested by changing pH, ionic strength and buffer composition. As a conclusion, we demonstrate that the LysM motif can be used as novel tag to purify recombinant proteins by affinity using an economical matrix, obtaining similar yields to the NiNTA system for protein purification.

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