Expression of keratinase gene in Bacillus megaterium using an expression vector of pHIS1525.SP_A and utilization of the resulting recombinant strain for chicken feather degradation prior to biogas production

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An increasing quantity of chickens is being utilized annually in the poultry industry, producing a huge volume of chicken feather waste which presents a high quality supply of keratin. Keratinases possessing high level of keratinolytic activity on insoluble keratin play a crucial role in hydrolyzing chicken feathers. Ever since the discovery of proteolytic ability as well as water solubility of keratinase, many industrial processes regarding keratinase application have been developed. A recently invented application to handle poultry waste is to utilize feathers for biogas production. Obviously, large amount of keratinase is required to break down the keratin prior to further conversion to biogas. Previously, several researches have shown that certain bacteria are able to produce keratinase but it is still a challenge to find out which bacteria is the most reliable source for the production with high efficiency. These challenges gave rise to the molecular biologists to bring the focus on gene cloning to develop recombinant strains resulting in overproduction of keratinase. Over the course of various cloning and expression experiments of similar proteins, it was found that Bacillus megaterium could be a susceptible host cell for keratinase production.

In our study, the keratinase gene from the chromosomal DNA of Bacillus licheniformis ATCC®53757 was PCR amplified and subsequently cloned into Bacillus megaterium expression vector, pKHERIS1525.SP_A. Bacillus megaterium ATCC®14945 strain was transformed with the recombinant plasmid, pKHERIS1525.SP_A. The keratinase gene was expressed under xylose inducible promoter, and the product was then purified using Ni-NTA affinity chromatography. After 18 h of incubation an extracellular keratinase activity of 29 U ml⁻¹ was achieved (one unit of activity was determined as the amount of enzyme required to an increase of 0.01 in A₄₂₀ after 30 min of incubation at 37°C). The recombinant strain was further examined for feather degradation using intact chicken feather waste as carbon source. The chopped chicken feathers were partially degraded by the recombinant strain after three days of incubation and the total macroscopic digestion was ultimately achieved after seven days resulting in a yellowish peptide rich fermentation broth. The biogas potential of the hydrolysate will be compared with that of untreated feathers by performing anaerobic batch digestion experiments.

Keywords: Bacillus licheniformis, Bacillus megaterium, Gene expression, Keratinase, Feather degradation, Biogas production

Fed-batch fermentation for heterologous protein production by recombinant Pichia pastoris

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Pichia pastoris is a methylotrophic yeast widely used for heterologous recombinant protein production. This yeast has potential for high level expression, efficient secretion and growth to very high cell densities. Fed-batch fermentation has been widely used to enhance protein production by P. pastoris. Frutalin is a α-D-galactose-binding lectin isolated from Artocarpus incisa seeds, successfully used as a cancer diagnostic tool and thus its large-scale production is aimed. This lectin has been previously expressed and produced in P. pastoris using a batch process. Therefore, the present work aims at evaluating a fed-batch fermentation process as an alternative to improve the production of recombinant frutalin by P. pastoris KM71H.

Cultivations were carried out in a 1.6 L reactor, in three distinct phases: 1) initial batch fermentation for cells growth in BMGH medium; 2) a fed-batch phase with 50% glycerol and 12 mL/L of trace metal solution; 3) a fed-batch phase where cells were induced by 0.5% methanol and 12 mL/L of trace metal solution. During the fermentation, the dissolved oxygen was kept above 30% saturation, aeration ratio was fixed at 1.5 vvm, and pH values were controlled at 5.0. In the first and second fermentation phases, the temperature was maintained at 30 °C, being decreased to 21 °C at the end of second stage. Total cell concentration was determined by measuring the absorbance of the broth at 600 nm, while glycerol consumption and methanol concentrations were detected by HPLC. The recombinant frutalin production was detected by denaturing SDS–PAGE, being the bands visualized by staining with Coomassie Brilliant Blue R250. The lectin activity was checked by hemagglutination assays towards rabbit red blood cells.

High cell density (98.8 g/L dry weight) was obtained during the fed-batch process (Fig. 1A), which is generally desirable since the concentration of secreted protein in the medium often increases proportionally to the cell density. Analysis by SDS–PAGE showed frutalin production at 120, 132 and 144 h. Native frutalin migrates in SDS–PAGE as a double band, where the upper band corresponds to the glycosylated isoforms and the lower band to the non-glycosylated isoforms. Recombinant frutalin migrated in gel as a single band (Fig. 1B) and exhibited hemagglutinating activity towards rabbit erythrocytes. Optimization of the induction phase is still on course. Nevertheless the results obtained so far show the feasibility of the fed-batch process for large-scale recombinant frutalin production by P. pastoris KM71H. Supported by: CNpq, ISAC, ERASMUS.

Keywords: Frutalin, Bacillus megaterium, Gene expression, Keratinase, Feather degradation, Biogas production

Fig. 1. Production of recombinant frutalin in fed-batch fermentation process by P. pastoris KM71H. A) Yeast growth profile and time course of batch, fed-batch and induction phases. B) SDS–PAGE analysis of the supernatant at induction phase.