Sudathip Titapoka 2007: Characterization of Mannanase from Effective Bacterial Strains and Their Application for Prebiotic Production. Doctor of Philosophy (Biotechnology), Major Field: Biotecnology, Department of Biotechnology. Thesis Advisor: Associate Professor Sunee Nitisinprasert, D.Sc. 129 pages.

In order to select the effective mannanase-producing bacterial strains to produce a mannooligosaccharide used as a prebiotic, two steps of screening were proposed. Forty-eight mannanase producing bacterial isolates from 3,055 isolates showing the activities of 1.33–3.0. The isolate CW2-3 showed the maximum activity of 3. While, only one fungi isolate from 1,079 isolates, SN2-1, could display the activity of 1.62. Only 10 isolates of CW1-2, CW2-1, CW2-3, ST1-1, ST2-2, ST3-4, ST3-10, BC-5 BC-7 and SN2-1 from 49 isolates showed specific mannanase activity of 0.054 – 0.731U/mg protein against copra meal. The bacterial isolate ST1-1 showed the highest mannanase activity of 0.290 U/ml. Copra meal hydrolysates (CM-hydrolysate) of isolate CW2-3 displayed the highest enhancing activity of *Lactobacillus reuteri* KUB-AC5 of 2.15 log(cfu/ml). Based on morphological, physical, biochemical and genetics properties, the isolate CW2-3 and ST1-1 were identified as *Klebsiella oxytoca* and *Acinetobacter* sp, respectively. The temperature and pH optimum of ST1-1 and CW2-3 were 40°C, 6 and 50°C, 7, respectively. The stability of mannanase from CW2-3 and ST1-1 exhibited a broad pH range of 3-6, and 3-10, respectively. Eighty-two percents of mannanase activity from CW2-3 still remained up to 60°C. While, the mannanase from ST1-1 was stable at 40-50°C for 30 min with relative activity around 95-100%. The mannanase S1 from *K. oxytoca* CW2-3 was purified about 99.23-fold with 3.15% recovery by anion exchange chromatography. Homogeneity of the S1 determined by 10% SDS-PAGE and 7.5% native-PAGE, the S1 was a single protein approximately of 165 kDa with pI of 3.5. The optimum pH of S1 was shown at pH 4.0. The stability of S1 exhibited a broad pH range of 3-6. The maximum activity of S1 was at 40°C. Remaining activity of 50% of S1 was stable at 40, 50, 60, 70°C for 4, 3, 3, 1h, respectively. In addition, the mannanase activity was completely inhibited by EDTA and Zn^{2+}, but activated by Co^{2+} (129%). The S1 exhibited high specific for galactomannan substrate, with no xylanase and cellulase activity detected. The Michealis-Menten constants (K_m), maximum velocity (V_{max}) and the catalytic constant (K_{cat}) values of S1 against LBG were 1.056 mg/ml, 6.149 μU/ml.min, and 0.047 Sec^{-1}, respectively. While, the K_m, V_{max} and k_{cat} values of S1 on Konjak mannan were 1.038 mg/ml, 6.183 μU/ml.min, and 0.047 Sec^{-1}, respectively. The first 15 N-terminal amino acid sequence (GRVGEAGPHGPHGPH) of the mannanase S1 was not similar to the N-terminal region of any bacterial mannanase in protein database. The degradation products from LBG hydrolysis of S1 were considered to be galactose, triose, tetraose and larger oligosaccharides than mannotetraose. While, the copra meal hydrolysis product contai ned the higher oligosaccharides. Both CM-hydrolysate and Cobalt treated CM-hydrolysate enhance and inhibit the growth of *Lactobacillus reuteri* KUB-AC5 and *Escherichia coli* E010 as well as *Salmonella* serovar Enteritidis S003, respectively.

Sudathip Titapoka — Student's signature

Thesis Advisor's signature

29 / Aug / 2007