

Maythiwan Kiatgrajai 2007: Preparation and Activity Test of Hydrolytic Enzymes from *Bacillus subtilis* GN156 for Napier Silage Quality Improvement. Master of Science (Biotechnology),
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Only 2.4-3.5 g/kg DM of water soluble carbohydrates were found in Napier grass. It affected low silage quality which were pH 4.3, 19.95 g/kg DM of lactic acid, 15.63 g/kg DM of acetic acid, 1.69 g/kg DM of ammonia nitrogen, 7.44 log CFU/g silage of lactic acid bacteria (LAB) and less than 1 log CFU/g of Enterobacteria, yeast and mold. Therefore, the objectives of this study were to improve silage quality by the use of hydrolytic enzymes from *Bacillus subtilis* GN156. Two different enzyme preparation, the E-NB and E-NM, were obtained from cell free culture fluid (CFS) of Nutrient broth culture with xylan as an inducer later concentrated by ammonium sulfate precipitation and from CFS of NM (g/l, dextrin, 20; yeast extract, 20; NaCl, 5; KH_2PO_4 , 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 and CaCl_2 , 0.1), respectively. The E-NB contained CMCase, pectinase, xylanase and beta-glucanase of 3.05, 71.46, 0.00 and 33.5 units/ml while the E-NM did of 464.05, 4799.45, 6167.82 and 13370.46 units/ml, respectively. They were different in both enzymatic system and quantity resulting in higher grass degradation of the E-NM (333.54 units/ml) than the one of the E-NB (33.50 units/ml) for 9.96 times. When 7.26 and 29.04 units/ml of E-NB were applied with lactic acid bacteria as additives, the reducing sugar contents of silage were higher than the control for 21.76 and 33.98 % in d 0 of ensiling, respectively. However these sugar content increments did not affect the lactic acid production during 0-3 d of ensiling. When the E-NM was applied, reducing sugar concentration of the E-NM treated silage were higher for 28.21 % of d 0 fermentation resulting in higher lactic acid concentration of 37.45 and 41.52 % of d 1 and 3 comparing to the E-NB treated silage. However both silage products showed no significant difference of the chemical and microbiological properties of the E-NM and the E-NB treated silage on d 60 of fermentation. When water soluble carbohydrates used as carbon source by LAB were analyzed by thin-layer chromatography, both the E-NB and E-NM treated silage of 4 h showed similar in both type and molecular weight oligosaccharide between trisaccharide and tetrasaccharide, as well as mono- and di-saccharide of ribose, arabinose, glucose, mannose, galactose, fructose and sucrose.

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