ABSTRACT

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ACTIVITY OF CRUDE PROTEASE FROM INDONESIAN Bacillus licheniformis MUTANTS F11.1 AND F11.4 ON FOOD ALLERGENS
(xiii + 87 pages, 12 figures; 8 tables, 11 appendices)

Crude protease obtained from two new mutants of Bacillus licheniformis F11.1 and F11.4 was obtained and studied for its hydrolysis action on three food allergens: Bos d8, Gal d1 and wheat gluten. Using SigmaAldrich (1999) assay, the effect of incubation period, hydrolysis temperature and pH was investigated. Both mutants showed highest protease activity at pH 9-10. F11.1 showed its highest activity at 50°C, while F11.4 had its highest activity at 50°-60°C. As for incubation period factor, F11.1 exhibited its highest proteolytic activity at hour 15 and hour 19 for F11.4. Extraction for Gal d1 was carried out with the use of TCA-acetone precipitation process and its solubilization was performed in 20 mM phosphate buffer pH 7.6. Wheat gluten was obtained through wet kneading and rinsing process with 2% NaCl solution, followed by solubilization in 0.25 M NaOH solution overnight. The protein concentration measured in ovomuoid and gluten was measured to be 5.82 mg/ml and 8.23 mg/ml respectively. SigmaAldrich (1999) assay was performed using casein, ovomucoid and wheat gluten as substrates at pH 10, 60°C, and with final concentration of all three allergens at 2.328 mg/ml. The result showed that the protease F11.1 showed its highest activity on gluten, while F11.4 exhibited highest hydrolysis action on ovomucoid and its least action on gluten. SDS-PAGE analysis on the hydrolyzed and unhydrolyzed allergens showed highest hydrolytic extent on casein while ovomucoid underwent the least changes. Fromzymography, crude protease from strain F11.1 was shown to contain one protease, which had the ability to hydrolyze casein and ovomucoid. Crude protease F11.4 contained four proteases in total. One of the proteases exhibited positive enzymatic action on casein, gluten and ovomucoid. Among the other three proteases, one was confirmed to be able to hydrolyze ovomucoid while the other two was shown positive for their ability to hydrolyze gluten.

Keyword: allergens, proteolytic, Bacillus licheniformis, ovomucoid, casein, gluten, electrophoresis, SDS-PAGE, zymography

References: 76 (1950-2010)