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Development of a rapid spectrophotometric method for bacterial mucinase

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Mucin, epithelial mucous glycoproteins, which line the respiratory and intestinal tracts of mammals, consists of repeating polypeptide sequences that are modified by oligosaccharide chains attached to serine or threonine residues. Mucin-producing cells often present easy-to-see borders, since the carbohydrate- rich pale-staining mucin contrasts with the protein-rich pink-staining cell surfaces. In previous study, we observed some correlation between the production of extracellular enzymes and the mouse lethality among various *Vibrio* species. Many studies have been suggested that the mucin is the critical factor for the initiation of pathogenic bacterial infection. However, the previously known detection method for mucin degradation is not only tedious and time-consuming process but the methods gave highly variable results. In this study, we developed a rapid and easy method for measuring the mucinase activity using bovine or porcine submaxillary mucin. The substrate mucin is cleaved between the oligosaccharide chain and the side chain of peptide linkage thereby liberating the N-acetyl neuraminic acid (NANA). The release of NANA increases the absorbance at 280 nm. *Vibrio parahaemolyticus* mucinase efficiently cleaved this substrate permitting continuous assay at NANA concentrations lower than 50 mM. At substrate concentrations between 25 to 200 mM, a good linearity was observed. A novel spectrophotometric assay was compared with periodate oxidation and PAS staining method for the assay of the mucinase produced from *V. parahaemolyticus*.