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Identification of a Novel Restriction Enzyme from *Helicobacter Pylori* that Recognizes and Clevages 5'-|GATC-3' / 3'-CTAG|-5'

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Strains of *Helicobacter pylori* are highly diverse at the individual gastric level. This high degree of genetic diversity may arise from various kinds of restriction and modification enzymes. More than 40 genes that may relate to restriction and modification are reported at each of whole genome sequence. Therefore *H. pylori* may maintain variegate restriction modification enzyme. We report here purification and characterization of a novel restriction enzyme from clinical isolate strain, Hp 206.

Hp206 cells were grown in Brucella Broth at 37℃ in microaerobic condition and harvested. The cells were disrupted by ultrasonication and cell debris was removed by ultracetrifugation. After removed DNA from crude extract by mixed with polyethyleneimine, we purified the enzyme with DEAE-sepharose column, Heparin-sepharose colum and Superrose column successively.

【Result】 ① The enzyme was partially purified and the specific recognition site was determined by comparison of cleavage pattern with known similar enzyme and by sequenceing as 5'-GATC-3'. ② We identified this restriciton enzyme clevage site as 5'↓-GATC-3'. ③ When the recognition sequence is modified by *dam* methylase (N6-adenin methyl transferase), the enzyme lost DNA clevage activity. ④ The enzyme required Mg^{2+} for cleaving the substrate DNA, but not ATP or S-adenosyl methionine. We consider therefore this restriction enzyme is categorized type II restriction enzyme. ⑤ The optimal NaCl concentration of the enzyme is 60 mM, which is lost at 100 mM, while that of R. *Mbo* I is 1000 mM.

【Conclusion】 We identified a novel restriction enzyme from Hp206. A gene in Hp that has homology with that of R. *Mbo* I may be responsible to express this enzyme.