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Identification of a new restriction endonuclease, R·*Ngo*BI, from *Neisseria gonorrhoeae*

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As a species, Neisseria gonorrhoeae produces five restriction endonucleases (1), and several other DNA methyltransferases (2). We have purified a methyltransferase that recognizes the sequence 5' TCACC 3' (2) and report here the purification from N. gonorrhoeae WR302 a restriction endonuclease, R·NgoBI, that also recognizes this sequence. Purification scheme: The purification scheme employed was as previously described (2) except the $(NH_4)_2SO_4$ precipitate was dissolved in buffer A (20 mM KPO_4, 1 mM EDTA, 10% glycerol, 10 mM 2-mercaptoethanol, pH 7.5) before being purified by chromatography through a 2 x 20 cm phosphocellulose column. The enzyme activity eluted at 0.15 M NaCl. Active fractions were further purified through an Accel QMA column and active fractions eluted at 0.1 M NaCl. The recognition sequence for R.NgoBI was determined by digesting lambda DNA with it and comparing the banding pattern obtained with computer generated patterns obtained with all known enzymes. The data indicated that this enzyme cleaved lambda DNA at the same sequence as HphI. Figure 1 is a comparison of the fragments obtained after digesting pUC8 with NgoBI and HphI. The restriction enzyme was most active in a buffer containing 25 mM Tris-HCl, 25 mM KCl, 10 mM $MgCl_2$, 2 mM 2-mercaptoethanol, pH 7.8.

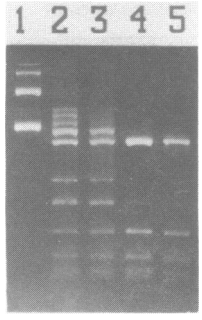

Figure 1. Digestion of pUC8 with NgoBI and HphI. The lanes represent 1) undigested DNA; 2) digested with NgoBI, 2 hr incubation; 3) digested with NgoBI, 3 hr incubation; 4) digested with NgoBI, 4 hr incubation; and 5) digested with HphI. Reactions were carried out in 28 µl and contained 2.5 µl of Accel QMA column eluate.

Contrary to what was previously thought, it appears that most DNA methyltransferases in N. gonorrhoeae are part of restriction/modification systems.

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References

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