Two novel restriction endonucleases from *Pseudomonas aeruginosa*

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*Pae*PI and *Pae*HI, type II restriction endonucleases have been isolated from clinical strain *Pseudomonas aeruginosa* 4148.

The enzymes were separated and purified by chromatography on DEAE-cellulose DE52, hydroxylapatite and mono Q column (FPLC system, Pharmacia Ltd).

*Pae*PI cleaves pBR322, pUC19 and M13mp8 at unique sites whose localizations corresponded to the *Pst*I site. A double digest between *Pae*PI and *Pst*I on bacteriophage lambda DNA confirmed these enzymes to be isoschizomers (Figure 1). The position of phosphodiester bond cleavage within the recognition site was determined by examination of a primed synthesis reaction. Sequencing reactions were performed as described by Sanger *et al.* (1). Samples were analyzed without or with further incubation with T4 DNAP and all four dNTPs by electrophoresis and subsequent autoradiography (Figure 2B). *Pae*PI was found to generated 3′ protruding TGCA-tetranucleotide. Thus, *Pae*PI and *Pst*I are true isoschisomers.

*Pae*HI cleaved pHSG415 (2) at unique site mapped near *Nru*I site in *neo* gene (Km^R), pUC19 at unique site within polylinker region and pBR322 at two sites within tetracycline resistance gene (data not shown). Comparison of these data and the *Pae*HI cleavage pattern on lambda DNA with computer-derived data predicted the sequence 5′-GRGCYC-3′. This suggestion was confirmed by double digest of lambda DNA with *Pae*HI and *Eco*241 which is the *Hgi*JII isoschisomer (3). The position of phosphodiester bond cleavage within the recognition site was determined by examination of a primed synthesis reaction. Sequencing reactions were performed as described by Sanger *et al.* (1). Samples were analyzed with or without further incubation with T4 DNAP and all four dNTPs by electrophoresis and subsequent autoradiography (Figure 2A). *Pae*HI was found to generated 3′ protruding PuGCPy-tetranucleotide. Thus, *Pae*HI and *Hgi*JII are true isoschisomers.

According to the recent list of restriction enzymes (3), *Pseudomonas aeruginosa* strains are poor sources of type II restriction endonucleases. The *Pst*I and *Hgi*JII specificities are new for this genus.

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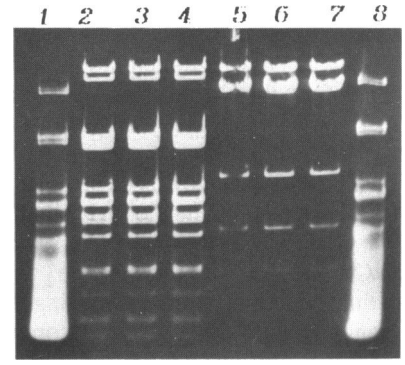

Figure 1. Digest of Lambda DNA: 2, *Pae*PI; 3, *Pst*I; 4, [ps8x]PaePI + *Pst*I; 5, *Pae*HI; 6, *Eco*241; 7, *Pae*HI + *Eco*241; 1,8, crude extract of *P.aeruginosa* 4148.

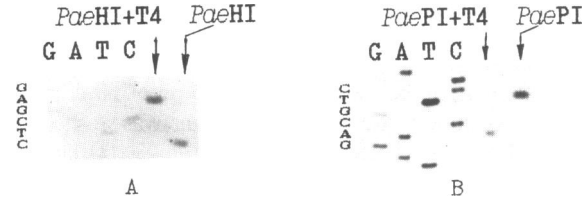

Figure 2. Determination of *Pae*HI (A) and *Pae*PI (B) cleavage positions.

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