Purification and characterization of *Asp*MDI, an isoschizomer of *Sau*3AI, from a marine bacterium, *Alcaligenes* sp MD1

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A new type II restriction endonuclease, *Asp* MD1, was isolated from a pigmented marine bacterium *Alcaligenes* species MD1 which was collected from the open sea around Taiwan Island. *Asp* MD1 is an isoschizomer of *Sau*3AI which recognizes four palindromic sequences 5'GATC3' (1). Cells were grown at 25°C up to full growth in a modified sea water broth (PPES II) containing 0.2% polypepton (Daigo Eiyo co. Ltd.), 0.1% proteose peptone No.3, 0.1% bacto yeast extract and 0.1% bacto soytone (Difco Laboratories) dissolved in artificial sea water (Jamarin Laboratory). The cell yield from 1 liter of culture broth was about 5–7 g in wet weight. Since non-specific nuclease activity of the cell lysate was significantly low, the enzyme was purified easily using CM- and DEAE-Sephadex chromatography. Restriction enzymic activity was determined at 30°C, in a reaction mixture containing 50 mM Tris-HCl, 5 mM β -mercaptoethanol, 10 mM $MgCl_2$, 200 mM NaCl and 1.0 μ g of substrate DNA, at pH 7.5. The optimum Mg^{+2} and Na^+ concentration, pH, and temperature conditions for *Asp* MD1 are summarized in Table 1 with the specific reference to *Sau*3AI (1). *Asp* MD1 exhibits a lower optimum temperature and higher Mg^{+2} concentration. Tolerance to salt concentration, which is considered a characteristic feature of marine bacterial enzymes, is also seen. The molecular weight of the enzyme was estimated by SDS-PAGE to be 66 K and/or 37 K daltons, where the molecular weight of *Sau*3AI was estimated to be 58 K and 35 K daltons, thus the monomer size of both enzymes is comparable. For the estimation of the recognition site, cleavage patterns of *Asp* MD1 derived from DNAs of T4dc, λ dam⁺ and dcm⁺, λ dam⁻, λ dcm⁻ pBR322 and ϕ X174RFI were compared with those produced by *Sau*3AI, and they were consistent with each other (Figure 1). The cleavage site of *Asp* MD1 was determined by cleavage of a primed synthesis reaction (2), and confirmed as shown in Figure 2.

REFERENCES

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Table 1. Restriction conditions for *Asp* MD1 with the specific reference to *Sau*3AI.

Enzyme	Temp. °C	pH	Mg^{+2} mM	NaCl mM	Max. Na^+ tolerance ^a , mM
<i>Asp</i> MD1	25–30	7.5	20	75	300
<i>Sau</i> 3AI	37	7.5	6	50	120

^aThe highest Na^+ concentration in which the enzyme retained 100% activity.

Figure 1. Comparison of the cleavage patterns of *Asp* MD1 and *Sau*3AI on λ dam⁺ and dcm⁺, λ dam⁻, λ dcm⁻, pBR322, ϕ X174RFI and T4dc. M, Marker λ /HindIII; 1, λ dam⁺ and dcm⁺; 2, λ dam⁺ and dcm⁺/*Sau*3AI; 3, λ dam⁺ and dcm⁺/*Asp* MD1; 4, λ dam⁻; 5, λ dam⁻/*Sau*3AI; 6, λ dam⁻/*Asp* MD1; 7, λ dcm⁻; 8, λ dcm⁻/*Sau*3AI; 9, λ dcm⁻/*Asp* MD1; 10, pBR322; 11, pBR322/*Sau*3AI; 12, pBR322/*Asp* MD1; 13, ϕ X174RFI; 14, ϕ X174RFI/*Sau*3AI; 15, ϕ X174RFI/*Asp* MD1; 16, T4dc; 17, T4dc/*Sau*3AI and 18, T4dc/*Asp* MD1.

Figure 2. An M13mp8 containing a *Bam*HI cleavage site was used for enzymatic sequencing reactions with an M13 universal primer I. The four standard dideoxy DNA sequencing reactions were done, and the fifth reaction containing no dideoxy termination was extended through the *Bam*HI site. The double-stranded DNA, thus extended, was used as substrate for *Asp* MD1. The cleaved product gave a single band (lane -), which co-migrated with 5' G in the sequence 5'GAT-CC3'. Following after the addition of Klenow a single band was produced which co-migrated with the 4th 5'C (lane +). These results indicate that *Asp* MD1, is an isoschizomer of *Sau*3AI.