SsbI, an isoschizomer of *Hind*III isolated from *Streptomyces scabies*

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*Ssb*I is a restriction endonuclease identified in cell lysates of the soil bacterium *Streptomyces scabies*, isolate FL1 (1). *Ssb*I was partially purified by DEAE−Sephadex chromatography to remove contaminating nuclease activity. The partially purified *Ssb*I fraction was used to determine the recognition sequence and the cleavage site for the endonuclease.

A single cleavage site was mapped for *Ssb*I in pACYC184 (2, 3). Double digests of *Ssb*I with *Eco*RI (Figure 1, Lane 1), *Nco*I (Figure 1, Lane 2), and *Sal*I (Figure 1, Lane 3) produced fragments whose lengths are consistent with *Ssb*I cleavage near the *Hind*III site in this plasmid. Control double digests of pACYC184 using *Hind*III with *Eco*RI, *Nco*I, and *Sal*I (Figure 1, Lanes 5, 6, and 7) generated fragment lengths similar to the *Ssb*I cleavage. Digestion of bacteriophage lambda (4) and pBR322 (5) with *Ssb*I also generated fragments consistent with cleavage near the *Hind*III site in these DNA molecules (data not shown). This data suggested that *Ssb*I recognized a sequence at or close to the sequence recognized by *Hind*III (6).

The cleavage site for *Ssb*I was determined by digestion of a primer extension product of pUC18 (7). A double stranded substrate was prepared as described previously (8) using reverse primer, ^{32}P-α-dATP, sequenase and pUC18 linearized with *Nde*I. In Figure 2 the lengths of the fragments generated by *Ssb*I digestion were determined by comparison of the *Ssb*I digest with the dideoxy-sequencing reactions of pUC18 using reverse primer. The fragment length aligned with the first A in the recognition sequence 5′A/AGCTT 3′ (Figure 2. Lane 1). When the product of *Ssb*I cleavage was filled-in with Klenow and dNTPs, the fragment length increased four nucleotides and corresponded to the first T of the recognition sequence (Figure 2, Lane 2). These results were identical to those obtained from the control reactions digested with *Hind*III (Figure 2, Lane 3, 4). Therefore, *Ssb*I is an isoschizomer of *Hind*III, with the same recognition sequence and cleavage site.

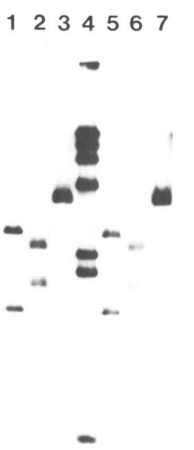

Figure 1. Restriction endonuclease cleavage of the pACYC184 plasmid with *Ssb*I. The plasmid, pACYC184, was digested with *Eco*RI (Lanes 1, 5), *Nco*I (Lanes 2, 6) or *Sal*I (Lanes 3, 7), and end-labelled by a fill-in reaction with Klenow and ^{32}P-α-dATP. The ^{32}P-labelled pACYC184 fragments were digested with *Ssb*I (Lanes 1−3) or *Hind*III (Lanes 5−7), electrophoresed on a 1% agarose gel and exposed to XOMAT film. A ^{32}P-labelled lambda *Hind*III digest was used as size marker (Lane 4).

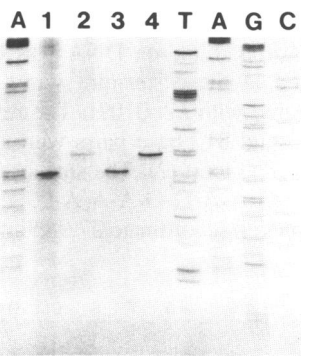

Figure 2. Identification of the *Ssb*I cleavage site in pUC18. The lanes A, G, C, and T, refer to standard dideoxy-chain termination reactions of pUC18 using reverse primer. In lanes 1−4, a ^{32}P-α-dATP labelled primer extension of *Nde*I linearized PUC18, initiated from the reverse primer, was used as a substrate for the reactions. The extension product was cleaved with *Ssb*I (Lane 1) or *Hind*III (Lane 3) to generate a 96 nt digestion product. The cleavage products were then treated with Klenow and dNTPs (Lane 2, *Ssb*I digest; Lane 4, *Hind*III digest) and generated a fragment 100 nt in length.

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