BavAI, a restriction endonuclease from *Bacillus alvei*

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A restriction endonuclease *BavAI*, free of contaminating nuclease activity was isolated from *Bacillus alvei* strain 675 using column chromatography on DEAE cellulose and Blue Sepharose CL-6B. Optimal conditions for *BavAI* digestion were determined at 30°C in a buffer containing 10 mM MgCl₂, 30 mM KCl at pH 7.5–8.3. The yield of the enzyme from 10 grams of wet cells was 22 000 units. The enzyme cleaves lambda phage DNA at 15 sites, Ad-2 DNA at 24 sites and plasmid pBR322 DNA at a unique site. The single cleavage site of *BavAI* on pBR322 DNA was mapped by standard double digestion analysis to approximately position 2000. From these data we deduced that the restriction enzyme cuts at *PvuII* sites. This was confirmed by comparing patterns of lambda DNA digests obtained with *BavAI*, *PvuII* and *BavAI* + *PvuII* simultaneously (Figure 1, lanes 1–3).

Sequence analysis of the *BavAI* cleavage site was performed on a pUC19 derivative with an insert containing *PvuII* site according to the enzymatic sequencing approach as described (1). Denatured ds DNA was used for dideoxy sequencing reactions starting with universal sequencing primer according to the quasi-terminal labeling procedure (2). In parallel reaction the labeled strand was extended by treatment with Klenow enzyme and four dNTPs and nascent ds DNA was used as substrate for *BavAI* to produce DNA fragments comparable to the sequencing ladder. Samples were analysed either with or without subsequent incubation with T4DNAP and all four dNTPs (Figure 2). The observed bands comigrated with G(5') in the recognition sequence 5'-CAGCTG-3' indicating that blunt-ended DNA fragments are produced.

From the mapping and sequencing data we conclude that *BavAI* is an isoschizomer of *PvuII* with specificity:

REFERENCES

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2. Kraev, A.S. and Mironov, V.N. (1990) *Molekularnaya biologia (Russ.)* **24**, 1095–1099.

Figure 1. 1% agarose gel of lambda DNA digests; lane 1, *BavAI*; lane 2, *PvuII*; lane 3, *BavAI* + *PvuII*; lane 4, *HindIII*.

Figure 2. *BavAI* cleavage site. After removing Klenow polymerase the extended nascent DNA template was cleaved with *BavAI* and run in parallel with standard sequencing reactions (lanes G,A,T,C) either with (lane [+]) or without (lane [-]) subsequent incubation with T4DNAP and all four dNTPs.