Isolation and properties of a new site-specific endonuclease \textit{Bmel42I} from \textit{Bacillus megaterium} 142

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A new type II restriction endonuclease, \textit{Bmel42I}, was partially purified and characterized from \textit{Bacillus megaterium} 142. It has been seen in a crude extract, because this bacterial strain does not have significant amounts of contaminating nucleases. The enzyme was purified by chromatography on phosphocellulose P11 (elution buffer - 10 mM K-phosphate, pH 7.0, 0.1 mM EDTA, 2 mM DTT, 0.2-1 M NaCl) and hydroxyapatite (elution buffer - 0.01-0.5 M K-phosphate, pH 7.0, 1 mM EDTA, 2 mM DTT). Yield of enzyme is 2000 units per gram of wet cells. The enzyme is stable in elution buffer and may be stored at -20\textdegree C after addition of glycerol to 50%. The recognition site was determined from the cleavage pattern of \textit{pBR322} plasmid. The 5'-end nucleotide is G. This has been shown by incubation of the 5'-end labelled DNA fragments with nuclease P1 followed by thin-layer chromatography on a PEI cellulose plate (1). The points of cleavage of the recognition site were determined by the Maxam-Gilbert method. In contrast to its isoschisomer, the restriction endonuclease \textit{HaeII}, which cleaves the same recognition site to produce a 4 base 3' extension, the \textit{Bmel42I} produces blunt-ended DNA fragments:

\begin{align*}
\text{5'} & \text{ PuGC+GCPy 3'} \\
\text{3'} & \text{ PyCG+CGPu 5'}
\end{align*}

\textbf{Fig. 1.} Didest of Lambda DNA: 
1 - \textit{Bmel42I},
2 - \textit{HaeII},
3 - \textit{Bmel42I} + \textit{HaeII}

\textbf{Fig. 2.} Sequencing gel of \textit{Cfr13I/NdeI} fragment of DNA \textit{pUC19}. Chemical modifications: 
1 - G; 2 - A+G; 3 - C; 4 - C+T; 5,6 - fragment digested \textit{Bmel42I} and \textit{HaeII}, respectively.

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\textbf{REFERENCES}