

P059 Creation of a DNA U/G endonuclease activity in Mma3148, an ExoIII homologue of the mesophilic archaeon *Methanosarcina mazei*

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Current research into DNA repair in archaea is predominantly directed towards organisms in the thermophilic to hyperthermophilic range of growth temperatures. We initiated a study of DNA-U repair in the mesophilic archaeon *Methanosarcina mazei*, for which a complete genomic sequence is available. Like all other archaea inspected so far, *M. mazei* does not possess a UDG family 1 representative. Instead, the genome contains two reading frames, the products of which qualify as candidates for initiating DNA-U repair: Mma0486 is a homolog of tUDGA (family 4) and Mma3148 is a homolog of AP-endonuclease ExoIII (earlier, we have shown that in *Methanothermobacter thermoautotrophicus*, DNA-U repair is mediated by ExoIII homolog Mth212). Genes Mma3148 and Mma0486 were cloned and their products characterised biochemically. Mma0486 exhibits all features of a family 4 uracil DNA glycosylase. Surprisingly, however, the enzyme maintains activity up to 77 °C. As expected for an ExoIII homolog, Mma3148 has AP-endonuclease and 5'→3' exonuclease activities. In addition, it displays U-endonuclease activity against U/Py but, unlike Mth212, not against U/G mispairs. We set out to graft this latter activity onto Mma3148. Guided by multiple sequence alignment and 3D structural considerations, we constructed double mutant Mma3148/A118M/M209Y and subsequently demonstrated U/G endonuclease activity associated with this variant.