Ago nucleases from Clostridium butyricum and Limnothrix rosea can process DNA substrates at moderate temperatures

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Prokaryotic Argonaute proteins (pAgos) are diverse homologs of eukaryotic Argonautes (eAgos) involved in RNA interference. In contrast to eAgos, which are RNA-guided RNA nucleases, several pAgos were reported to act as DNA nucleases suggesting that they may be used as an alternative to CRISPR-Cas nucleases for genome editing. However, all previously studied pAgos were isolated from thermophilic bacteria or archaea thus limiting their potential use in genomic applications. We describe two pAgo nucleases from mesophilic bacteria, *Clostridium butyricum* (CbAgo) and *Limnothrix rosea* (LrAgo). Both CbAgo and LrAgo use small DNA guides to cleave complementary DNA targets and are active at physiological temperatures. At the same time, the two proteins reveal significant variations in DNA processing depending on the reaction conditions and guide structure, including changes in the guide 5'-end and the presence of mismatches. We show that CbAgo is highly active under a wide range of conditions and can precisely cleave single-stranded and double-stranded DNA at moderate temperatures suggesting that it may be used for DNA manipulations both *in vitro* and *in vivo*. This work was supported by the grant 14.W03.31.0007 of the Ministry of Science and Higher Education of the Russian Federation.