from >60°C to 80°C with hyperthermophiles capable of optimal growth above 80°C), for example, microorganisms that are used in industrial fermentations or for *in vitro* laboratory processes conducted at elevated temperatures.

To date there is no experimental evidence for active Cas9 proteins in thermophiles. Based on a comparative genome screening by Chylinski *et al.* (2014; Nucleic Acids Research 42: 6091-61-05) on the presence of Cas9 in bacteria it was found that the Type II-C CRISPR-Cas system is only present in approximately 3.3% of all bacterial genomes. Among thermophilic bacteria, the Type II system is underrepresented based on statistical analysis (P=0.0019). In addition, no Type II system has been found in archaea however, this could possibly be due to the absence of the RNase III protein (involved in the Type II system) in archaea. Chylinski, *et al.*, (2014; Nucleic Acids Research 42: 6091-6105) did describe the classification and evolution of type II CRISPR-Cas systems, in particular, two species are identified which exhibit these systems, however these species grow maximally at 55°C and do not exhibit strictly thermophilic growth with optimum growth temperature 60-80°C, with hyperthermophiles capable of growing optimally above 80°C.

Despite the rarity of the CRISPR-Cas system in bacterial genomes and in particular the fact that Cas9 has been found only in bacteria (not archaea) with optimal growth temperatures below 45°C, the inventors have surprisingly discovered several thermostable Cas9 variants which enable genome editing to be carried out at elevated temperatures. These Cas9 nucleases provide novel tools for genetic engineering at elevated temperatures and are of particular value in the genetic manipulation of thermophilic organisms; particularly microorganisms.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an isolated Cas protein or polypeptide fragment, having an amino acid sequence of SEQ ID NO: 1 or a sequence of at least 77% identity therewith, wherein the Cas protein is capable of cleaving a polynucleotide comprising a target nucleic acid sequence between 50°C and 100°C, when associated with at least one RNA molecule which recognizes the target sequence.

In another aspect, the present invention provides a ribonucleoprotein complex comprising a Cas protein as described herein, and comprising at least one targeting RNA molecule which recognises a sequence in a target polynucleotide.

In yet another aspect, the present invention provides an isolated nucleic acid molecule encoding a clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) protein having an amino acid sequence of SEQ ID NO: 1 or a sequence of at least 77% identity therewith; or a polypeptide fragment thereof.

In yet another aspect, the present invention provides an expression vector comprising a nucleic acid molecule as described herein.

In yet another aspect, the present invention provides a method of modifying a target nucleic acid comprising contacting the nucleic acid with:

a. a ribonucleoprotein complex as described herein; or

b. a protein or protein complex as described herein and at least one targeting RNA molecule as described herein.

In yet another aspect, the present invention provides a method of modifying a target nucleic acid in a cell, comprising transforming, transfecting or transducing the cell with an expression vector as described herein; or alternatively transforming, transfecting or transducing the cell with an expression vector as described herein and a further expression vector comprising a nucleotide sequence encoding a targeting RNA molecule as described herein.

In yet another aspect, the present invention provides a method of modifying a target nucleic acid in a cell comprising transforming, transfecting or transducing the cell with an expression vector as described herein, and then delivering a targeting RNA molecule as described herein to, or into the cell.

In yet another aspect, the present invention provides a method of silencing gene expression at a desired locus according to any of the methods as described herein.

In yet another aspect, the present invention provides a method of modifying or deleting and/or inserting a desired nucleotide sequence at a desired location according to any of the methods as described herein.

In yet another aspect, the present invention provides a host cell transformed by a method as described herein.

DESCRIPTION

Accordingly, the present invention provides an isolated clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) protein or polypeptide comprising;

a. the amino acid motif EKDGKYYC [SEQ ID NO: 2]; and/or
b. the amino acid motif $X_1X_2CTX_3X_4$ [SEQ ID NO: 3] wherein X_1 is independently selected from Isoleucine, Methionine or Proline, X_2 is