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 disclosure provides a ribonucleoprotein complex comprising a Cas protein having an amino acid sequence of SEQ ID NO: 1, or a polypeptide fragment thereof, and at least one targeting RNA molecule which recognises a target nucleic acid sequence in a targetDNA, wherein the ribonucleoprotein complex is capable of cleaving said target DNA comprising the target nucleic acid sequence at a temperature of 65°C, for cleaving or modifying genetic material of an organism, and wherein the targeting RNA molecule is a single guide RNA (sgRNA).

In another aspect, the present disclosure provides an expression vector comprising a polynucleotide sequence encoding a Cas protein having an amino acid sequence of SEQ ID NO: 1, or a polypeptide fragment thereof, and a sequence encoding a selectable marker.

In another aspect, the present disclosure provides an expression vector comprising a polynucleotide sequence encoding the ribonucleoprotein complex as disclosed herein.

In yet another aspect, the present disclosure provides a method of modifying a target nucleic acid comprising contacting the nucleic acid *in vitro* with:

a. the ribonucleoprotein complex as described herein; or

b. an isolated Cas protein comprising an amino acid sequence of SEQ ID NO: 1, or a polypeptide fragment thereof, and at least one targeting RNA molecule as disclosed herein.

In yet another aspect, the present disclosure provides an *in vitro* method of modifying a target nucleic acid in a cell, comprising (a) transforming, transfecting or transducing the cell with an expression vector as described herein and with a nucleotide sequence encoding a targeting RNA molecule which recognises a target nucleic acid sequence in the cell; or (b) 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 which recognises a target nucleic acid sequence in the cell; or (c) transforming, transfecting or transducing the cell with an expression vector as disclosed herein.

In yet another aspect, the present disclosure provides an *in vitro* 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 disclosure provides a host cell comprising a polynucleotide sequence encoding the ribonucleoprotein complex as disclosed herein or the expression vector as disclosed herein, transformed by a method as disclosed herein.

DESCRIPTION

Accordingly, the present invention provides an isolated clustered regularly interspaced short palindromic repeat (CRISpPR)-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

CLAIMS

1. A ribonucleoprotein complex comprising a Cas protein having an amino acid sequence of SEQ ID NO: 1, or a polypeptide fragment thereof, and at least one targeting RNA molecule which recognises a target nucleic acid sequence in a target DNA, wherein the ribonucleoprotein complex is capable of cleaving said target DNA comprising the target nucleic acid sequence at a temperature of 65 °C, for cleaving or modifying genetic material of an organism, and wherein the targeting RNA molecule is a single guide RNA (sgRNA).

2. The ribonucleoprotein complex as claimed in claim 1, wherein the length of the at least one targeting RNA molecule is in the range 35 – 135 nucleotide residues.

3. The ribonucleoprotein complex as claimed in claim 1 or claim 2, wherein the target nucleic acid sequence is 31 or 32 nucleotide residues in length.

4. The ribonucleoprotein complex as set forth in any one of claims 1 to 3, wherein the Cas protein or polypeptide fragment is provided as part of a protein complex comprising at least one further protein.

5. The ribonucleoprotein complex as claimed in claim 4, wherein the Cas protein or polypeptide fragment, and/or the at least one further protein comprise at least one functional moiety.

6. The ribonucleoprotein complex as claimed in any one of claims 1 to 3, wherein the native nuclease activity of the Cas protein or polypeptide fragment is inactive and the Cas protein or polypeptide fragment is fused to at least one functional moiety.

7. The ribonucleoprotein complex as claimed in claim 6, wherein the at least one functional moiety is fused or linked to the N-terminus and/or the C-terminus of the Cas protein or polypeptide fragment thereof; preferably the N-terminus.

8. The ribonucleoprotein complex as claimed in any one of claims 5 to 7, wherein the at least one functional moiety is a protein; optionally selected from a helicase, a nuclease, a helicase-nuclease, a DNA methylase, a histone methylase, an acetylase, a phosphatase, a kinase, a transcription (co-)activator, a transcription repressor, a DNA binding protein, a DNA structuring protein, a marker protein, a

reporter protein, a fluorescent protein, a ligand binding protein, a signal peptide, a subcellular localisation sequence, an antibody epitope or an affinity purification tag.

9. The ribonucleoprotein complex as claimed in claim 8, wherein the native nuclease activity of the Cas protein or polypeptide fragment is inactive and the at least one functional moiety is a nuclease domain; preferably a FokI nuclease domain.

10. The ribonucleoprotein complex as claimed in claim 8, wherein the native nuclease activity of the Cas protein or polypeptide fragment is inactive and the at least one functional moiety is a marker protein.

11. The ribonucleoprotein complex as claimed in any one of claims 1 to 10, when used for cleaving or modifying genetic material *in vitro*.

12. An expression vector comprising a polynucleotide sequence encoding a Cas protein having an amino acid sequence of SEQ ID NO: 1, or a polypeptide fragment thereof, and a sequence encoding a selectable marker.

13. An expression vector comprising a polynucleotide sequence encoding the ribonucleoprotein complex as defined in any one of claims 1 to 11.

14. A method of modifying a target nucleic acid comprising contacting the nucleic acid *in vitro* with:

a. the ribonucleoprotein complex as set forth in any one of claims 1 to 11; or

b. an isolated Cas protein comprising an amino acid sequence of SEQ ID NO: 1, or a polypeptide fragment thereof, and at least one targeting RNA molecule as defined in claim 1.

15. An *in vitro* method of modifying a target nucleic acid in a cell, comprising (a) transforming, transfecting or transducing the cell with an expression vector of claim 12 and with a nucleotide sequence encoding a targeting RNA molecule which recognises a target nucleic acid sequence in the cell; or (b) transforming, transfecting or transducing the cell with an expression vector of claim 12 and a further expression vector comprising a nucleotide sequence encoding a targeting RNA molecule which recognises a target nucleic acid sequence in the cell; or (c)

transforming, transfecting or transducing the cell with an expression vector of claim 13.

16. An *in vitro* method of modifying a target nucleic acid in a cell comprising transforming, transfecting or transducing the cell with an expression vector of claim 12 and then delivering a targeting RNA molecule as defined in claim 1 to, or into, the cell.

17. The *in vitro* method of modifying a target nucleic acid as claimed in any one of claims 14 to 16, wherein the ribonucleoprotein complex or isolated Cas protein comprises a marker protein or reporter protein and the marker protein or reporter protein associates with the target nucleic acid; preferably wherein the marker is a fluorescent protein, for example a green fluorescent protein (GFP).

18. The *in vitro* method as claimed in any one of claims 14 to 17, wherein the target nucleic acid is DNA; preferably dsDNA.

19. The *in vitro* method as claimed in any one of claims 14 to 17, wherein the target nucleic acid is RNA.

20. The *in vitro* method of modifying a target nucleic acid as claimed in claim 18, wherein the nucleic acid is dsDNA, the ribonucleoprotein complex or isolated Cas protein comprises a nuclease or a helicase-nuclease, and the modification is a single-stranded or a double-stranded break at a desired locus.

21. The *in vitro* method as claimed in any one of claims 15, 16, 18 or 20, for silencing gene expression at a desired locus.

22. The *in vitro* method as claimed in any one of claims 15, 16, 18 or 20, for modifying or deleting and/or inserting a desired nucleotide sequence at a desired location.

23. The *in vitro* method as claimed in any one of claims 15, 16, 18 or 20, for modifying gene expression in a cell comprising modifying a target nucleic acid sequence; wherein the target nucleic acid is dsDNA and the ribonucleoprotein complex or isolated Cas protein comprises a DNA modifying enzyme (e.g. a methylase or acetylase), a transcription activator or a transcription repressor.

24. The *in vitro* method as claimed in claim 19, wherein the nucleic acid is an mRNA and the ribonucleoprotein complex or isolated Cas protein comprises a

ribonuclease; optionally selected from an endonuclease, a 3' exonuclease or a 5' exonuclease.

25. The *in vitro* method of modifying a target nucleic acid as claimed in any one of claims 14 to 24, wherein the method is carried out at a temperature between 50°C and 100°C.

26. The *in vitro* method of modifying a target nucleic acid as claimed in claim 25, wherein the method is carried out at a temperature at or above 60°C, preferably between 60°C and 80°C, more preferably between 60°C and 65°C.

27. The method as claimed in any one of claims 14 to 26, wherein the cell is a prokaryotic cell.

28 The method as claimed in any one of claims 14 to 26, wherein the cell is a eukaryotic cell.

29. A host cell comprising a polynucleotide sequence encoding the ribonucleoprotein complex as defined in any one of claims 1 to 11, or the expression vector of claim 12 or claim 13, transformed by a method as claimed in any of claims 14 to 28.

30. The host cell as claimed in claim 29, wherein the nucleic acid molecule encoding the Cas protein or polypeptide is fused to at least one nucleic acid sequence encoding a protein selected a helicase, a nuclease, a helicase-nuclease, a DNA methylase, a histone methylase, an acetylase, a phosphatase, a kinase, a transcription (co-)activator, a transcription repressor, a DNA binding protein, a DNA structuring protein, a marker protein, a reporter protein, a fluorescent protein, a ligand binding protein, a signal peptide, a subcellular localisation sequence, an antibody epitope or an affinity purification tag.