comprising a GGDEF motif and an I-site wherein at least one of the conserved amino acids, arginine or aspatic acid of the I-site is replaced with a neutral amino acid forming a mutant diguanylate cyclase that does not bind c-di-GMP.

[0011] Preferably the neutral amino acid is either a non-polar amino acid or an uncharged polar amino acid. In one embodiment the non-polar amino acid is alanine. In one embodiment the uncharged polar amino acid is glutamine.

[0012] In a preferred embodiment the diguanylate cyclase polypeptide comprises an amino acid sequence at least 90% homologous to a diguanylate cyclase polypeptide comprising a GGDEF motif isolated from a thermophilic bacteria. Preferably the thermophilic bacteria is selected from *Thermotoga maritima; Thermoanaerobacter ethanolicus; Thermoanaerobacter tengcongensis; Fervidobacterium nodosum; Thermosipho melanesiensis; thermotoga petrophila*; or *Moorella thermoacetica*. In one emdodiment the thermophilic bacteria is *Thermotoga maritima.*

[0013] Preferably the mutant diguanylate cyclase comprises a monomer in solution.

[0014] In a preferred embodiment the mutant diguanylate cyclase comprises a sequence of SEQ ID NO.: 2 or SEQ ID NO. 3, or SEQ ID NO. 3

[0015] In one ~~emdodiment~~ embodiment the diguanylate cyclase is coupled to a support. In one embodiment the support is a sol-gel.

[0016] Another aspect of the invention provides a polynucleotide capable of expressing a mutant diguanylate cyclase polypeptide comprising a GGDEF motif and an I-site wherein at least one of the conserved amino acids, arginine or aspatic acid of the I-site is replaced with a neutral amino acid. ~~In o~~One embodiment ~~the polynucleotide of claim 10 comprising~~ includes the sequence of SEQ ID NO. 1.

[0017] Another aspect of the invention provides an expression vector comprising The polynucleotide of the invention operably linked to a regulatory sequence capable of directing expression of said polynucleotide in a host cell.

We claim:

1. A diguanylate cyclase polypeptide according to a sequence selected from the group consisting of SEQ ID NO.:2, SEQ ID NO.:3, and SEQ ID NO.:4.~~with improved thermostability comprising a GGDEF motif and an I-site wherein at least one of the conserved amino acids, arginine or aspartic acid of the I-site is replaced with a neutral amino acid forming a mutant diguanylate cyclase that does not bind c-di-GMP.~~

~~2. The diguanylate cyclase polypeptide of claim 1 wherein the neutral amino acid is a non-polar amino acid.~~

~~3. The diguanylate cyclase polypeptide of claim 2 wherein the non-polar amino acid is alanine.~~

~~4. The diguanylate cyclase polypeptide of claim 1 wherein the neutral amino acid is an uncharged polar amino acid.~~

~~5. The diguanylate cyclase polypeptide of claim 4 wherein the uncharged polar amino acid is Glutamine.~~

~~6. The diguanylate cyclase polypeptide of claim 1 or 2 comprising an amino acid sequence at least 90% homologous to a diguanylate cyclase polypeptide comprising a GGDEF motif isolated from a thermophilic bacteria.~~

~~7. The diguanylate cyclase polypeptide of claim 6 wherein the thermophilic bacteria is selected from *Thermotoga maritima; Thermoanaerobacter ethanolicus; Thermoanaerobacter tengcongensis; Fervidobacterium nodosum; Thermosipho melanesiensis; thermotoga petrophila*; or *Moorella thermoacetica*.~~

~~8. The diguanylate cyclase polypeptide of claim 6 wherein the thermophilic bacteria is *Thermotoga maritima*.~~

~~9.~~2. The diguanylate cyclase polypeptide of ~~any one of~~ claims 1 ~~to 8~~ wherein the ~~mutant~~ diguanylate cyclase comprises a monomer in solution.

~~10. The diguanylate cyclase polypeptide of any one of claims 1 to 8 wherein the mutant diguanylate cyclase comprises SEQ ID NO.: 2.~~

~~11. The diguanylate cyclase polypeptide of any one of claims 1 to 8 wherein the mutant diguanylate cyclase comprises SEQ ID NO. 3.~~

~~12. The diguanylate cyclase polypeptide of any one of claims 1 to 8 wherein the mutant diguanylate cyclase comprises SEQ ID NO. 4.~~

~~13.~~3. The diguanylate cyclase polypeptide of claim 1 ~~any one of the preceding claims~~ wherein the ~~mutant~~ diguanylate cyclase is coupled to a support.

~~14.~~4. The diguanylate cyclase polypeptide of claims ~~13~~ 3 wherein the support is a sol-gel.

~~15. A polynucleotide capable of expressing a mutant diguanylate cyclase polypeptide with improved thermostability comprising a GGDEF motif and an I-site wherein at least one of the conserved amino acids, arginine or aspartic acid of the I-site is replaced with a neutral amino acid.~~

~~16. The polynucleotide of claim 15 comprising the sequence of SEQ ID NO. 1.~~

~~17. An expression vector comprising the polynucleotide of claims 15 or 16 operably linked to a regulatory sequence capable of directing expression of said polynucleotide in a host cell.~~

~~18.~~5. The diguanylate cyclase polypeptide of ~~any one of~~ claims 1 ~~to 14~~ wherein the ~~mutant~~ diguanylate cyclase polypeptide is synthesized chemically.

~~19. The diguanylate cyclase polypeptide of any one of claims 1 to 14 wherein the mutant diguanylate cyclase polypeptide is produced in the expression vector of claim 17.~~

~~20.~~6. A method of manufacturing cyclic-di-GMP or cyclic-di-GMP analogues comprising the steps of:

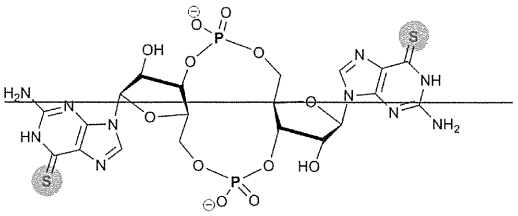
a. incubating the ~~mutant~~ diguanylate cyclase polypeptide of ~~any one of~~ claims 1 ~~to 14~~ with Guanosine-5'-triphosphate; and

b. Isolating the cyclic-di-GMP or cyclic-di-GMP analogues.

~~21.~~7. The method of claim 6 ~~20~~ wherein the analogue comprises 6-thio cyclic-di-GMP.

~~22.~~8. The method of claim ~~20~~6 wherein the analogue comprises 32P-radioisotope-labelled cyclic-di-GMP.

~~23.An analogue manufactured by the method of claim 20 comprising the formula:~~



comprising a GGDEF motif and an I-site wherein at least one of the conserved amino acids, arginine or aspatic acid of the I-site is replaced with a neutral amino acid forming a mutant diguanylate cyclase that does not bind c-di-GMP.

[0011] Preferably the neutral amino acid is either a non-polar amino acid or an uncharged polar amino acid. In one embodiment the non-polar amino acid is alanine. In one embodiment the uncharged polar amino acid is glutamine.

[0012] In a preferred embodiment the diguanylate cyclase polypeptide comprises an amino acid sequence at least 90% homologous to a diguanylate cyclase polypeptide comprising a GGDEF motif isolated from a thermophilic bacteria. Preferably the thermophilic bacteria is selected from *Thermotoga maritima; Thermoanaerobacter ethanolicus; Thermoanaerobacter tengcongensis; Fervidobacterium nodosum; Thermosipho melanesiensis; thermotoga petrophila*; or *Moorella thermoacetica*. In one emdodiment the thermophilic bacteria is *Thermotoga maritima.*

[0013] Preferably the mutant diguanylate cyclase comprises a monomer in solution.

[0014] In a preferred embodiment the mutant diguanylate cyclase comprises a sequence of SEQ ID NO.: 2 or SEQ ID NO. 3, or SEQ ID NO. 3

[0015] In one embodiment the diguanylate cyclase is coupled to a support. In one embodiment the support is a sol-gel.

[0016] Another aspect of the invention provides a polynucleotide capable of expressing a mutant diguanylate cyclase polypeptide comprising a GGDEF motif and an I-site wherein at least one of the conserved amino acids, arginine or aspatic acid of the I-site is replaced with a neutral amino acid. One embodiment includes the sequence of SEQ ID NO. 1.

[0017] Another aspect of the invention provides an expression vector comprising the polynucleotide of the invention operably linked to a regulatory sequence capable of directing expression of said polynucleotide in a host cell.

We claim:

1. A diguanylate cyclase polypeptide according to a sequence selected from the group consisting of SEQ ID NO.:2, SEQ ID NO.:3, and SEQ ID NO.:4.
2. The diguanylate cyclase polypeptide of claim 1 wherein the diguanylate cyclase comprises a monomer in solution.
3. The diguanylate cyclase polypeptide of claim 1 wherein the diguanylate cyclase is coupled to a support.
4. The diguanylate cyclase polypeptide of claim 3 wherein the support is a sol-gel.
5. The diguanylate cyclase polypeptide of claim 1 wherein the diguanylate cyclase polypeptide is synthesized chemically.
6. A method of manufacturing cyclic-di-GMP or cyclic-di-GMP analogues comprising the steps of:
    a. incubating the diguanylate cyclase polypeptide of claim 1 with Guanosine-5'-triphosphate; and
    b. Isolating the cyclic-di-GMP or cyclic-di-GMP analogues.
7. The method of claim 6 wherein the analogue comprises 6-thio cyclic-di-GMP.
8. The method of claim 6 wherein the analogue comprises 32P-radioisotope-labelled cyclic-di-GMP.