Title: FTSZ MULTIMERIC PROTEINS AND THEIR USES

Abstract

The invention provides multimeric FtsZ polypeptides and polynucleotides encoding multimeric FtsZ polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are methods for utilizing multimeric FtsZ polypeptides to screen for antibacterial compounds.
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FTSZ MULTIMERIC PROTEINS AND THEIR USES

RELATED APPLICATIONS

This application claims benefit of US Provisional Patent Application Number 60/091,680, filed July 2, 1998.

FIELD OF THE INVENTION

This invention relates to newly identified higher order structures of FtsZ polypeptides, such as dimers, trimers, tetramers and larger aggregations, and their production and uses, as well as their variants, their agonists and antagonists, and their uses. In particular, the invention relates to multimeric polypeptides comprising FtsZ subunits and their use in screening for antimicrobial compounds.

BACKGROUND OF THE INVENTION

In the prokaryotic cell cycle, the FtsZ (filamentation temperature sensitive) protein functions early in the septation process. FtsZ polymerizes in a dynamic ring structure and localizes to the midpoint of the dividing cell. This polymeric structure is believed to act as a scaffold for other important cell division proteins. In addition to its structural role, FtsZ is also a GTPase. Prior to the Applicants findings provided herein, the precise relationship between GTPase activity of FtsZ and the polymerization-depolymerization cycle is poorly understood.


Clearly, there is a need for factors, such as the compounds of the invention, that have a present benefit of being useful to screen compounds for antibiotic activity. Such factors are also useful to determine their role in pathogenesis of infection, dysfunction and disease. There is also a need for identification and characterization of such factors and their antagonists and agonists which can play a role in preventing, ameliorating or correcting infections, dysfunctions or diseases.

SUMMARY OF THE INVENTION

The present invention relates to FtsZ multimeric and higher order forms of FtsZ, particularly FtsZ from Streptococcus pneumoniae, and Staphylococcus aureus, among others. In another aspect, the invention relates to methods for using such polypeptides, including the treatment of
microbial diseases, amongst others. In a further aspect, the invention relates to methods for identifying agonists and antagonists using the materials provided by the invention, and for treating microbial infections and conditions associated with such infections with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting the presence or activity of multimeric FtsZ polypeptides.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 illustrates data from a malachite green assay that indicates the hydrolysis of GTP by FtsZ.

Figure 2 illustrates data from a centrifugation experiment demonstrating the existence of FtsZ dimers and tetramers.

Figure 3 illustrates data showing a temperature dependence relationship in FtsZ dimers and tetramer association.

Figure 4 illustrates data showing concentration dependence of FtsZ self-association.

Figure 5 illustrates data showing FtsZ self-association in the presence of GDP.

Figure 6 illustrates data showing FtsZ self-association in the presence of gS-GTP.

**DETAILED DESCRIPTION OF THE INVENTION**

The Applicants have demonstrated, utilizing both analytical ultracentrifugation and enzymatic assays, an important relationship between FtsZ GTPase activity and oligomeric state of FtsZ protein, by showing that FtsZ dimers, trimers and/or tetramers are required for expression of GTP hydrolysis activity. In view of this discovery, phosphate release assays, such as the malachite green or MEG coupled enzyme assay, among other assays provided herein, will be used to identify compounds that interfere with FtsZ self-recognition, particularly those compounds that prevent GTP hydrolysis. Such compounds are believed by the Applicants to be broad spectrum antibacterial agents since it is known that FtsZ is an essential bacterial cell division protein that is present in all known bacterial species.

The invention relates to multimeric FtsZ polypeptides as described in greater detail below. In particular, the invention relates to polypeptides of a multimeric FtsZ, particularly of *Streptococcus pneumoniae*, each monomeric subunit of which is related by amino acid sequence homology to *Lactococcus lactis* FtsZ U74322 polypeptide. The invention relates especially to multimeric FtsZ each subunit comprising the amino acid sequences set out in Table 1 as SEQ ID NO: 2 or 4.
## TABLE 1

### Monomeric FtsZ Polypeptide Sequences

(A) *Streptococcus pneumoniae* monomeric FtsZ polypeptide [SEQ ID NO:2].

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<tr>
<th>Sequence</th>
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<tr>
<td>MLEFEQGFLALVOLKLQVGGGGNNVRMDHMNNVFIAINTDGQALNLSKAESKIQIGIKEKTRGLG</td>
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<td>SHRKRSGSTGFTSVNTSNNATSKDESFSTNNSQATDSVSTERTHTTKEDIFSFIINREERSRRTRR</td>
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(B) *Streptococcus pneumoniae* polynucleotide encoding the monomeric FtsZ polypeptide of [SEQ ID NO:2 [SEQ ID NO:1].

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<td>CTAGCCATTTAGAATAGAAGAAAGAGACGCTTCAAGAGAACCAAAGACGTTAA</td>
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Monomeric FtsZ Polypeptide Sequences

(C)  Staphylococcus aureus monomeric FtsZ polypeptide [SEQ ID NO:4].

MTSFSDTAAGQAVIKGVGGGAGAARTDVMETGVSTEMTAAATDVQALSSTKAETVQLGFKLTRLG
GAGQPEVGR
KAAESEETLTEASGADMFIITAGGGSSTGAAVPVIALIADGLGALTGVVTRPFPFEGSKRGQFAVE
GINQLREHVD
10 TLLIISNNNLLEIVVKTPPLLEALSEADNVLRQQVQGITTDLITNPNPLNLDFADVPVMAKGNALMGIG
IGSVEERVVE
AARKAIYSLLETTIDAGAEHNVTVGGLDLILITLEEASEASQIVNQAAGQVNIWLGTSIDEISMEIRVT
VVTATGVRQDR
VEKVPVQARSATNYRETVPKAHSHGFDRHFMDAETVELPKQNFKRRLEPTQASAFGDWDLRFERSIVRTD
15 SVSVSPVERFE
APISIQEDELDPFPEFKNR

(D)  Staphylococcus aureus polynucleotide encoding the monomeric FtsZ polypeptide of SEQ ID NO:4 [SEQ ID NO:3].

ATGACATTTCTATGATACGCTGCTCAAGGGCAGTAGTTAAAGTAATGGTGTCGTTGGAGGTG
GTGGCATTGC
CATCAACGCCTAGGCTGAGCAAGGTGATGAGTCAGCAGCAGAATATCAGTCGCAACAGCAAGCAT
TTGAGTATGA
20 CAAAGAAGCTGACGTGTTACATCATTGATGGACCTAAAATGACCTCGTGGTTGAGTTCAGGCTAAC
CTGATTTAG
GCTTTGCGTTACGCCAGCTGTCGCTCGTCTGTCTTATGCTGATGTGCTATGCCAAAAGATTAGTG
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TTAGGAGAC
30 CACGTCCCTTTTGGTTTGAAGAGAAGTAGACCTGGGACATTTTGCTGTAGAAGGAATCATACTACCTG
TGCACTATAGAC
35 GAGCCTATTCCCTTCTAGCTTTCCAGACCTTACCATACCTCTTCCATTACCCCGAGTTTACATTAC
ACCATCGAGTTGATTAACCTTT
40 GACTTTGCGG
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AGTGTGTTAGAA
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ACGGTTCTGG
45 TGAGAGAAACGGTATGGCTGCACCGTAAATGGCATGCTCTGCTCTAATACATAACCCGTGGCAAGCAT
GAGATGAAACGA
TCAAGACCCG
GTTGAGAAAGATTGTTGGCTCAACAACAGCTGATAGATCGCTCTACTAATACCGTGGAGACGATGAAC
ACAGCTATT
50 CCCAAATTTCCACAAGATGGAAAGATTGGATACACCTTTCTCATTCTCTCATTTCACAAATCGTTAA
Deposited materials

A deposit containing a *Streptococcus pneumoniae* 0100993 strain has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. (herein "NCIMB"), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland on 11 April 1996 and assigned deposit number 40794. The deposit was described as *Streptococcus pneumoniae* 0100993 on deposit. On 17 April 1996 a *Streptococcus pneumoniae* 0100993 DNA library in E. coli was similarly deposited with the NCIMB and assigned deposit number 40800. The *Streptococcus pneumoniae* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

The deposited strain contains the full length FtsZ gene. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

A license may be required to make, use or sell the deposited strain, and compounds derived therefrom, and no such license is hereby granted.

In one aspect of the invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressible by the *Streptococcus pneumoniae* 0100993 strain, which polypeptide is contained in the deposited strain. Further provided by the invention are FtsZ polynucleotide sequences in the deposited strain, such as DNA and RNA, and amino acid sequences encoded thereby. Also provided by the invention are multimeric FtsZ polypeptides comprising the FtsZ polypeptide sequences isolated from the deposited strain and the polynucleotides that encode such sequences.

**Polypeptides**

The multimeric FtsZ polypeptides of the invention is substantially phylogenetically related to other proteins of the FtsZ family.

In one aspect of the invention there are provided polypeptides, particularly of *Streptococcus pneumoniae* multimeric FtsZ polypeptides as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.
Among the particularly preferred embodiments of the invention are variants of multimeric FtsZ polypeptide each subunit of which is encoded by naturally occurring alleles of the FtsZ gene.

The present invention further provides for an isolated polypeptide which:
(a) comprises or consists of a multimeric FtsZ polypeptide each subunit of which is an amino acid sequence which has at least 75% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2 or 4 over the entire length of SEQ ID NO:2 or 4 respectively;
(b) a multimeric FtsZ polypeptide each subunit of which is a polypeptide encoded by an isolated polynucleotide comprising or consisting of a polynucleotide sequence which has at least 75% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:2 or 4 over the entire length of SEQ ID NO:2 or 4 respectively;
(c) a multimeric FtsZ polypeptide each subunit of which is a polypeptide encoded by an isolated polynucleotide comprising or consisting of a polynucleotide sequence encoding a polypeptide which has at least 75% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2 or 4, over the entire length of SEQ ID NO:2 or 4 respectively.

The multimeric FtsZ polypeptide include multimers each subunit of which comprises a polypeptide of Table 1 [SEQ ID NO:2 or 4] (in particular the mature polypeptide) as well as polypeptides and fragments, particularly those which have the biological activity of FtsZ and constitute a multimeric FtsZ having the biological activity of multimeric FtsZ, and also those which have at least 75% identity to a polypeptide of Table 1 [SEQ ID NO:2 or 4] or the relevant portion, preferably at least 80% identity to a polypeptide of Table 1 [SEQ ID NO:2 or 4] and more preferably at least 90% identity to a polypeptide of Table 1 [SEQ ID NO:2 or 4] and still more preferably at least 95% identity to a polypeptide of Table 1 [SEQ ID NO:2 or 4] and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

The invention also includes a multimeric FtsZ polypeptide each subunit of which is a polypeptide consisting of or comprising a polypeptide of the formula:

\[ X-(R_1)_m-(R_2)-(R_3)_n-Y \]

wherein, at the amino terminus, X is hydrogen, a metal or any other moiety described herein for modified polypeptides, and at the carboxyl terminus, Y is hydrogen, a metal or any other moiety described herein
for modified polypeptides, R₁ and R₃ are any amino acid residue or modified amino acid residue, m is an integer between 1 and 1000 or zero, n is an integer between 1 and 1000 or zero, and R₂ is an amino acid sequence of the invention, particularly an amino acid sequence selected from Table 1 or modified forms thereof. In the formula above, R₂ is oriented so that its amino terminal amino acid residue is at the left, covalently bound to R₁, and its carboxy terminal amino acid residue is at the right, covalently bound to R₃. Any stretch of amino acid residues denoted by either R₁ or R₃, where m and/or n is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer. Other preferred embodiments of the invention are provided where m is an integer between 1 and 50, 100 or 500, and n is an integer between 1 and 50, 100, or 500.

It is most preferred that a polypeptide of the invention is derived from Streptococcus pneumoniae, however, it may preferably be obtained from other organisms of the same taxonomic genus. A polypeptide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

A fragment is a variant polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any polypeptide of the invention.

Preferred fragments comprising a multimeric FtsZ include, for example, truncation polypeptides having a portion of an amino acid sequence of Table 1 [SEQ ID NO:2 or 4], or of variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell, particularly a Streptococcus pneumoniae, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO: 2 or 4, or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO: 2 or 4.

Also preferred are biologically active fragments which are those fragments that mediate activities of multimeric FtsZ once combined in multimeric form, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those fragments that are antigenic or immunogenic in an animal, especially in a human. Particularly preferred are fragments.
comprising receptors or domains of enzymes that confer a function essential for viability of *Streptococcus pneumoniae* or the ability to initiate, or maintain cause Disease in an individual, particularly a human.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention.

**Vectors, Host Cells, Expression Systems**

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems which comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention.

Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci *E. coli*, streptomyces, cyanobacteria, *Bacillus subtilis*, and *Streptococcus pneumoniae*; fungal cells, such as cells of a yeast, *Kluyveromyces*, *Saccharomyces*, a basidiomycete, *Candida albicans* and *Aspergillus*; insect cells such as cells of *Drosophila S2* and *Spodoptera Sf9*; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example,
vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, (supra).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

**Diagnostic, Prognostic, Serotyping and Mutation Assays**

This invention is also related to the use of multimeric FtsZ polypeptides of the invention for use as diagnostic reagents. Detection of multimeric FtsZ polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the multimeric FtsZ protein, may be detected by a variety of well known techniques, for example, by non-denaturing protein gels, as well as by methods provided herein.

Polypeptides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials.
In another embodiment, an array of antibodies against multimeric FtsZ can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification.

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

(a) a multimeric FtsZ polypeptide of the present invention, preferably a multimeric FtsZ polypeptide comprising at least one polypeptide of SEQ ID NO:2 or 4 or a fragment thereof; or
(b) an antibody to a multimeric FtsZ polypeptide of the present invention, preferably a multimeric FtsZ polypeptide comprising at least one polypeptide of SEQ ID NO:2 or 4.

It will be appreciated that in any such kit, (a) or (b) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a Disease, among others.

Cells from an organism carrying mutations or polymorphisms (allelic variations) in a multimeric FtsZ polypeptide of the invention may also be detected at the polypeptide level by a variety of techniques. For example, a diagnostic assay in accordance with the invention for detecting over-expression of multimeric FtsZ polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, among other diseases. Assay techniques that can be used to determine levels of a multimeric FtsZ polypeptide, in a sample derived from a host, such as a bodily material, are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection and ELISA assays.

**Antagonists and Agonists - Assays and Molecules**

Multimeric FtsZ polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, e.g., Coligan et al., *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

Multimeric FtsZ polypeptides of the present invention are responsible for or involved in many biological functions, such as bacterial cell division and bacterial cell survival. In view of the fact that such multimeric FtsZ polypeptides carry important bacterial biological functions, they are therefore responsible for the maintenance of many disease states, in particular the Diseases hereinbefore mentioned. It is therefore desirable to devise screening methods to identify compounds which stimulate or which inhibit the function of a multimeric FtsZ polypeptide and lead to slowing or stopping of bacterial growth or bacterial killing. Accordingly, in a further aspect, the present invention provides for
a method of screening compounds to identify those which stimulate or which inhibit the function of a multimeric FtsZ polypeptide of the invention, as well as related polypeptides. In general, agonists or antagonists may be employed for therapeutic and prophylactic purposes for such Diseases as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such agonists, antagonists or inhibitors so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of multimeric FtsZ polypeptides; or may be structural or functional mimetics thereof (see Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991)).

The screening methods may simply measure the binding of a candidate compound to a multimeric FtsZ polypeptide or polynucleotide, or to cells or membranes bearing such polypeptide, or a fusion protein of the polypeptide by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of a multimeric FtsZ polypeptide, using detection systems appropriate to the cells comprising such polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active multimeric FtsZ polypeptide and/or constitutively expressed polypeptides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of multimeric FtsZ polypeptide. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a multimeric FtsZ polypeptide of the present invention, to form a mixture, measuring multimeric FtsZ polypeptide activity in the mixture, and comparing the multimeric FtsZ polypeptide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and multimeric FtsZ polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett et al., J Mol Recognition, 8:52-58 (1995); and K. Johanson et al., J Biol Chem, 270(16):9459-9471 (1995)). As used herein "activity" means any functional attribute of a FtsZ polypeptide or multimeric FtsZ polypeptide, including, for example, enzymatic activity (such as GTPase activity), binding of ligands, substrates (such as GTP) or subunits (such as FtsZ polypeptide), the formation of multimers, the disassociation of multimers, cell division, cell growth ring formation and cell wall invagination, among others.
Multimeric FtsZ polypeptides and antibodies that bind to and/or interact with such polypeptides of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production multimeric FtsZ polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues. Methods of measuring the ability of a candidate compound to alter the assembly state of FtsZ are also preferred as screening methods of the invention.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of multimeric FtsZ polypeptides, particularly those compounds that are bacteriostatic and/or bacteriocidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising multimeric FtsZ polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a multimeric FtsZ agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the multimeric FtsZ polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, i.e., without inducing the effects of multimeric FtsZ polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in multimeric FtsZ polypeptide activity, and binding assays known in the art.

Multimeric FtsZ polypeptides of the invention may be used to identify membrane bound or soluble receptors, if any, for such polypeptide, through standard receptor binding techniques known in the art. These techniques include, but are not limited to, ligand binding and crosslinking assays in which the polypeptide is labeled with a radioactive isotope (for instance, $^{125}$I), chemically modified (for instance, biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (e.g., cells, cell membranes, cell supernatants, tissue extracts, bodily materials). Other methods include biophysical techniques...
such as surface plasmon resonance and spectroscopy. These screening methods may also be used to identify agonists and antagonists of the polypeptide which compete with the binding of the polypeptide to its receptor(s), if any. Standard methods for conducting such assays are well understood in the art.

A preferred assay to screen for antimicrobial compounds using multimeric FtsZ polypeptide is to add a test compound to multimeric FtsZ and determine compounds that antagonize the FtsZ:FtsZ interaction by measuring the amount of multimeric FtsZ over after the addition of a test compound. Compounds that antagonize the FtsZ:FtsZ interaction are believed to block the turning on of FtsZ GTPase activity. Moreover, compounds that affect or inhibit the FtsZ:FtsZ interaction are believed to affect the rate of GTP hydrolysis.

Preferred assay conditions for achieving GTPase activity and/or the formation of multimeric FtsZ FtsZ monomers or lower order forms are: 50mM Hepes, 50mM KCl, 5mM MgCl, pH 7.4.

A number of assays can be used to characterize compounds that block the FtsZ self-association and/or compounds that promote FtsZ self-association. The interaction between FtsZ monomers can be monitored with physical techniques such as analytical ultracentrifugation, fluorescence polarization, fluorescence energy transfer, surface plasmon resonance, scintillation proximity assay and the use of ion channel switch (ICS) biosensors, among other well known detection techniques.

Analytical ultracentrifugation is a practical and robust method for measuring molecular mass in solution. It may be used to demonstrate that FtsZ undergoes a monomer-dimer-tetramer or assembly process in the absence of FtsZ active site ligands or compounds that otherwise bind or interact with FtsZ. While not wanting to be limited to theoretical models, the Applicants believe that the midpoints of both assembly processes are at about 10 micromolar FtsZ, so the technique is ideally suited to quantitatively monitor changes in association that might occur as a result of the addition of small molecule inhibitors. The use of interference optics is preferred and allows these experiments to be done, irrespective of the absorption or fluorescence properties of the inhibitors.

The fluorescence polarization value for a fluorescently-tagged molecule depends on the rotational correlation time or tumbling rate. Large protein complexes, such as multimeric FtsZ, labeled to comprise a fluorescently-labelled molecule will have higher polarization values than a fluorescently labelled monomeric protein. It is preferred that this method be used to characterize small molecules that disrupt FtsZ oligomers.
Fluorescence energy transfer may be used to characterize small molecules that interfere with the formation of FtsZ dimers, trimers, tetramers or higher order structures. FtsZ can be labelled with both a donor and acceptor fluorophore. Upon mixing of the two labelled species and excitation of the donor fluorophore, fluorescence energy transfer can be detected by observing fluorescence of the acceptor. Compounds that block dimerization will inhibit fluorescence energy transfer.

Surface plasmon resonance can be used to monitor the effect of small molecules on FtsZ forms by self-association. FtsZ can be coupled to a sensor chip at low site density such that covalently bound molecules will be monomeric. Solution protein can then passed over the FtsZ-coated surface and specific binding can be detected in real-time by monitoring the change in resonance angle caused by a change in local refractive index. This technique can be used to characterize the effect of small molecules on kinetic rates and equilibrium binding constants for FtsZ forms by self-association.

A scintillation proximity assay may be used to characterize the interaction between FtsZ monomers. FtsZ can be coupled to a scintillation-filled bead. Addition of radio-labelled FtsZ results in binding where the radioactive source molecule is in close proximity to the scintillation fluid. Thus, signal is emitted upon FtZ binding and compounds that prevent FtsZ self-association will diminish signal.

ICS biosensors have been described by AMBRI (Australian Membrane Biotechnology Research Institute). They couple the self-association of macromolecules to the closing of gramicidin-facilitated ion channels in suspended membrane bilayers and hence to a measurable change in the admittance (similar to impedance) of the biosensor. This approach is linear over six decades of admittance change and is ideally suited for large scale, high through-put screening of small molecule combinatorial libraries.

In other embodiments of the invention there are provided methods for identifying compounds which bind to or otherwise interact with and inhibit or activate an activity or expression of a multimeric FtsZ polypeptide of the invention comprising: contacting a polypeptide of the invention with a compound to be screened under conditions to permit binding to or other interaction between the compound and the polypeptide to assess the binding to or other interaction with the compound, such binding or interaction preferably being associated with a second component capable of providing a detectable signal in response to the binding or interaction of the polypeptide with the compound; and determining whether the compound binds to or otherwise interacts with and activates or inhibits an activity or expression of the
polypeptide and/or polynucleotide by detecting the presence or absence of a signal generated from the binding or interaction of the compound with the polypeptide.

A preferred assay embodiment comprises the step of contacting an organism with a test compound and detecting if FtsZ forms a septal ring using an antibody of the invention. Compounds that are associated with disaggregation of such ring are preferred as antimicrobial compounds.

Another example of an assay for multimeric FtsZ agonists is a competitive assay that combines multimeric FtsZ and a potential agonist with multimeric FtsZ-binding molecules, recombinant multimeric FtsZ binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. The multimeric FtsZ molecule can be labeled, such as by radioactivity or a colorimetric compound, such that the number of multimeric FtsZ molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Rayleigh light scattering is an absolute method for determining the molecular weight of macromolecules in aqueous solution. Changes in light scattering that result from addition of an external compound reflect changes in the weight average molecular weight and hence in the assembly state, i.e. the relative amounts of monomers, dimers, tetramers and higher order assemblies. This technique could be used to identify compounds which reduce the weight average molecular weight, shifting the molecular distribution towards monomer, and hence acting as an inhibitor of FtsZ.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing multimeric FtsZ-induced activities, thereby preventing the action or expression of multimeric FtsZ polypeptides by excluding multimeric FtsZ polypeptides from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, J. Neurochem. 56: 560 (1991); OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of multimeric FtsZ. Other examples of potential polypeptide antagonists include antibodies or, in some cases, oligonucleotides or
proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Certain of the polypeptides of the invention are biomimetics, functional mimetics of the natural multimeric FtsZ polypeptide. These functional mimetics may be used for, among other things, antagonizing the activity of multimeric FtsZ polypeptide or as a antigen or immunogen in a manner described elsewhere herein. Functional mimetics of the polypeptides of the invention include but are not limited to truncated polypeptides. For example, preferred functional mimetics include, a multimeric FtsZ each subunit comprising a polypeptide comprising the polypeptide sequence set forth in SEQ ID NO:2 or 4 lacking 20, 30, 40, 50, 60, 70 or 80 amino- or carboxy-terminal amino acid residues, including fusion proteins comprising one or more of these truncated sequences. Polynucleotides encoding each of these functional mimetics may be used as expression cassettes to express each mimic polypeptide. It is preferred that these cassettes comprise 5' and 3' restriction sites to allow for a convenient means to ligate the cassettes together when desired. It is further preferred that these cassettes comprise gene expression signals known in the art or described elsewhere herein.

Thus, in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for a polypeptide and/or polynucleotide of the present invention; or compounds which decrease or enhance the production of such polypeptides and/or polynucleotides, which comprises:

(a) a multimeric FtsZ polypeptide of the present invention;
(b) a recombinant cell expressing a multimeric FtsZ polypeptide of the present invention;
(c) a cell membrane expressing a multimeric FtsZ polypeptide of the present invention; or
(d) antibody to an multimeric FtsZ polypeptide of the present invention;

which polypeptide is preferably that of SEQ ID NO:2 or 4.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

It will be readily appreciated by the skilled artisan that a polypeptide of the present invention may also be used in a method for the structure-based design of an agonist, antagonist or inhibitor of a multimeric FtsZ polypeptide, by:

(a) determining in the first instance the three-dimensional structure of the polypeptide, or complexes thereof;
(b) deducing the three-dimensional structure for the likely reactive site(s), binding site(s) or motif(s) of an agonist, antagonist or inhibitor;
(c) synthesizing candidate compounds that are predicted to bind to or react with the deduced binding site(s), reactive site(s), and/or motif(s); and
(d) testing whether the candidate compounds are indeed agonists, antagonists or inhibitors.

It will be further appreciated that this will normally be an iterative process, and this iterative process may be performed using automated and computer-controlled steps.

In a further aspect, the present invention provides methods of treating abnormal conditions such as, for instance, a Disease, related to either an excess of, an under-expression of, an elevated activity of, or a decreased activity of multimeric FtsZ polypeptide.

If the expression and/or activity of the polypeptide is in excess, several approaches are available. One approach comprises administering to an individual in need thereof an inhibitor compound (antagonist) as herein described, optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function and/or expression of the polypeptide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of the polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous polypeptide may be administered. Typical examples of such competitors include fragments of the multimeric FtsZ polypeptide.

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

In still another approach, expression of the gene encoding endogenous multimeric FtsZ polypeptide can be inhibited using expression blocking techniques. This blocking may be targeted against any step in gene expression, but is preferably targeted against transcription and/or
translation. An examples of a known technique of this sort involve the use of antisense sequences, either internally generated or separately administered (see, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Alternatively, oligonucleotides which form triple helices with the gene can be supplied (see, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360). These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

In accordance with yet another aspect of the invention, there are provided multimeric FtsZ polypeptide agonists and antagonists, preferably bacteriostatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.

*Helicobacter pylori* (herein "*H. pylori*") bacteria infect the stomachs of over one-third of the world’s population causing stomach cancer, ulcers, and gastritis (International Agency for Research on Cancer (1994) *Schistosomes, Liver Flukes and Helicobacter Pylori* (International Agency for Research on Cancer, Lyon, France, http://www.uicc.ch/ecp/ecp2904.htm)). Moreover, the International Agency for Research on Cancer recently recognized a cause-and-effect relationship between *H. pylori* and gastric adenocarcinoma, classifying the bacterium as a Group I (definite) carcinogen. Preferred antimicrobial compounds of the invention (agonists and antagonists of multimeric FtsZ polypeptides and/or polynucleotides) found using screens provided by the invention, or known in the art, particularly narrow-spectrum antibiotics, should be useful in the treatment of *H. pylori* infection. Such treatment should decrease the advent of *H. pylori*-induced cancers, such as gastrointestinal carcinoma. Such treatment should also prevent, inhibit and/or cure gastric ulcers and gastritis.

**Vaccines**

There are provided by the invention, products, compositions and methods for assessing multimeric FtsZ expression, treating disease, assaying genetic variation, and administering a multimeric FtsZ polypeptide and/or polynucleotide to an organism to raise an immunological response against a bacteria, especially a *Streptococcus pneumoniae* bacteria.

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal which comprises inoculating the individual with multimeric FtsZ polypeptide, or a fragment or variant thereof, adequate to produce antibody and/or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Streptococcus pneumoniae* infection. Also provided are methods whereby
such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of multimeric FtsZ polypeptide, or a fragment or a variant thereof, for expressing multimeric FtsZ polypeptide, or a fragment or a variant thereof in vivo in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a multimeric FtsZ polypeptide, wherein the composition comprises a recombinant multimeric FtsZ polypeptide. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A multimeric FtsZ polypeptide or a fragment thereof may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from Hemophilus influenzae, Glutathione-S-transferase (GST) or beta-galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. et al. Science 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof, which have been shown to encode non-variable regions of bacterial
cell surface proteins, in polynucleotide constructs used in such genetic immunization experiments in animal models of infection with *Streptococcus pneumoniae*. Such experiments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value, derived from the requisite organ of the animal successfully resisting or clearing infection, for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Streptococcus pneumoniae* infection, in mammals, particularly humans.

A polypeptide of the invention may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused, for example, by mechanical, chemical, thermal or radiation damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, throat, mammary glands, urethra or vagina.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant polypeptide and/or polynucleotide of the invention together with a suitable carrier, such as a pharmaceutically acceptable carrier. Since the polypeptides and polynucleotides may be broken down in the stomach, each is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostatic compounds and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

While the invention has been described with reference to certain multimeric FtsZ polypeptides, it is to be understood that this covers fragments of the naturally occurring polypeptides, and similar polypeptides and polynucleotides with additions, deletions or
substitutions which do not substantially affect the immunogenic properties of the recombinant polypeptides or polynucleotides.

Compositions, kits and administration

In a further aspect of the invention there are provided compositions comprising a multimeric FtsZ polypeptide for administration to a cell or to a multicellular organism.

The invention also relates to compositions comprising a polynucleotide and/or a polypeptides discussed herein or their agonists or antagonists. The polypeptides and polynucleotides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide and/or polynucleotide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such as the soluble form of a polypeptide and/or polynucleotide of the present invention, agonist or antagonist peptide or small
molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides, polynucleotides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, and the like.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

In-dwelling devices include surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters.

The composition of the invention may be administered by injection to achieve a systemic effect against relevant bacteria shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections, especially Streptococcus pneumoniae wound infections.

Many orthopedic surgeons consider that humans with prosthetic joints should be considered for antibiotic prophylaxis before dental treatment that could produce a bacteremia. Late deep infection is a serious complication sometimes leading to loss of the prosthetic joint and is
accompanied by significant morbidity and mortality. It may therefore be possible to extend the use of the active agent as a replacement for prophylactic antibiotics in this situation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, the composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1 μg/ml to 10mg/ml for bathing of wounds or indwelling devices.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

GLOSSARY

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"Activity" means any functional attribute of a FtsZ polypeptide or multimeric FtsZ polypeptide, including, for example, enzymatic activity (such as GTPase activity), binding of ligands, substrates (such as GTP) or subunits (such as FtsZ polypeptide), the formation of multimers, the disassociation of multimers, cell division, cell growth ring formation and cell wall invagation, among others.

“Antibody(ies)” as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.
"Antigenically equivalent derivative(s)" as used herein encompasses a polypeptide, polynucleotide, or the equivalent of either which will be specifically recognized by certain antibodies which, when raised to the protein, polypeptide or polynucleotide according to the invention, interferes with the immediate physical interaction between pathogen and mammalian host.

"Bispecific antibody(ies)" means an antibody comprising at least two antigen binding domains, each domain directed against a different epitope.

"Bodily material(s) means any material derived from an individual or from an organism infecting, infesting or inhabiting an individual, including but not limited to, cells, tissues and waste, such as, bone, blood, serum, cerebrospinal fluid, semen, saliva, muscle, cartilage, organ tissue, skin, urine, stool or autopsy materials.

"Disease(s)" means any disease caused by or related to infection by a bacteria, including, for example, otitis media, conjunctivitis, pneumonia, bacteremia, meningitis, sinusitis, pleural empyema and endocarditis, and most particularly meningitis, such as for example infection of cerebrospinal fluid.

"FtsZ" means, as the case may be, FtsZ polypeptides, FtsZ polynucleotides, each including, for example, those from any organism defined herein.

"Fusion protein(s)" refers to a protein encoded by two, often unrelated, fused genes or fragments thereof. In one example, EP-A-0464 discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for use in therapy and diagnosis resulting in, for example, improved pharmacokinetic properties [see, e.g., EP-A 0232262]. On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified.

"Host cell(s)" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polynucleotide sequence.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic

Parameters for polypeptide sequence comparison include the following:


Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:


Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the
reference sequence of SEQ ID NO:1 or 3, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or 3 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 or 3 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1 or 3, or:

\[ n_n \leq x_n - (x_n \cdot y), \]

wherein \( n_n \) is the number of nucleotide alterations, \( x_n \) is the total number of nucleotides in SEQ ID NO:1 or 3, \( y \) is 0.50 for 50\%, 0.60 for 60\%, 0.70 for 70\%, 0.80 for 80\%, 0.85 for 85\%, 0.90 for 90\%, 0.95 for 95\%, 0.97 for 97\% or 1.00 for 100\%, and \( \cdot \) is the symbol for the multiplication operator, and wherein any non-integer product of \( x_n \) and \( y \) is rounded down to the nearest integer prior to subtracting it from \( x_n \). Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 or 4 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100\% identity to a polypeptide reference sequence of SEQ ID NO:2 or 4, wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or 4 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in
SEQ ID NO:2 or 4 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2 or 4, or:

\[ n_a \leq x_a - (x_a \cdot y) \]

wherein \( n_a \) is the number of amino acid alterations, \( x_a \) is the total number of amino acids in SEQ ID NO:2 or 4, \( y \) is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and \( \cdot \) is the symbol for the multiplication operator, and wherein any non-integer product of \( x_a \) and \( y \) is rounded down to the nearest integer prior to subtracting it from \( x_a \).

"Immunologically equivalent derivative(s)" as used herein encompasses a polypeptide, polynucleotide, or the equivalent of either which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the immediate physical interaction between pathogen and mammalian host.

"Immunospecific" means that characteristic of an antibody whereby it possesses substantially greater affinity for the polypeptides of the invention or the polynucleotides of the invention than its affinity for other related polypeptides or polynucleotides respectively, particularly those polypeptides and polynucleotides in the prior art.

"Individual(s)" means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

"Isolated" means altered "by the hand of man" from its natural state, \( i.e., \) if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Multimeric FtsZ" means a dimeric protein, trimeric protein, tetrameric protein or higher order structure protein comprising FtsZ protein subunits. Mutlimeric FtsZ may be comprised of FtsZ proteins of different species or amino acid sequences as provided by the invention and described herein.

"Organism(s)" means a (i) prokaryote, including but not limited to, a member of the genus Streptococcus, Staphylococcus, Bordetella, Corynebacterium, Mycobacterium, Neisseria,
Haemophilus, Actinomycetes, Streptomyces, Nocardia, Enterobacter, Yersinia, Fancisella, Pasteurella, Moraxella, Acinetobacter, Erysipelothrix, Branhamella, Actinobacillus, Streptobacillus, Listeria, Calymmatobacterium, Brucella, Bacillus, Clostridium, Treponema, Escherichia, Salmonella, Klebsiella, Vibrio, Proteus, Erwinia, Borrelia, Leptospira, Spirillum, Campylobacter, Shigella, Legionella, Pseudomonas, Aeromonas, Rickettsia, Chlamydia, Borrelia and Mycoplasma, and further including, but not limited to, a member of the species or group, Group A Streptococcus, Group B Streptococcus, Group C Streptococcus, Group D Streptococcus, Group G Streptococcus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus faecalis, Streptococcus faecium, Streptococcus durans, Neisseria gonorrhoeae, Neisseria meningitidis, Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium diphtheriae, Gardnerella vaginalis, Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium ulcerans, Mycobacterium leprae, Actinomyces israelii, Listeria monocytogenes, Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica, Escherichia coli, Shigella dysenteriae, Haemophilus influenzae, Haemophilus aegyptius, Haemophilus parainfluenzae, Haemophilus ducreyi, Bordetella, Salmonella typhi, Citrobacter freundii, Proteus mirabilis, Proteus vulgaris, Yersinia pestis, Klebsiella pneumoniae, Serratia marcescens, Serratia liquefaciens, Vibrio cholera, Shigella dysenteriae, Shigella flexneri, Pseudomonas aeruginosa, Franciscella tularensis, Brucella abortis, Bacillus anthracis, Bacillus cereus, Clostridium perfringens, Clostridium tetani, Clostridium botulinum, Treponema pallidum, Rickettsia rickettsii and Chlamydia trachomatis, (ii) an archaeon, including but not limited to Achaebacter, and (iii) a unicellular or filamentous eukaryote, including but not limited to, a protozoan, a fungus, a member of the genus Saccharomyces, Kluyveromyces, or Candida, and a member of the species Saccharomyces cerevisae, Kluyveromyces lactis, or Candida albicans.

"Polynucleotide(s)" generally refers to any polynucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotide(s)" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions or single-, double- and triple-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded regions, or a mixture of single- and double-stranded regions. In addition, "polynucleotide" as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term "polynucleotide(s)" also includes
DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotide(s)" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide(s)" as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells. "Polynucleotide(s)" also embraces short polynucleotides often referred to as oligonucleotide(s).

"Polypeptide(s)" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene encoded amino acids.

"Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphoethanolamine, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in
POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed.,
(1992). Polypeptides may be branched or cyclic, with or without branching. Cyclic, branched and
branched circular polypeptides may result from post-translational natural processes and may be made by
entirely synthetic methods, as well.

"Recombinant expression system(s)" refers to expression systems or portions thereof or
polynucleotides of the invention introduced or transformed into a host cell or host cell lysate for the
production of the polynucleotides and polypeptides of the invention.

"Subtraction set" is one or more, but preferably less than 100, polynucleotides
comprising at least one polynucleotide of the invention

"Variant(s)" as the term is used herein, is a polynucleotide or polypeptide that differs from
a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical
variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide.
Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of
a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino
acid substitutions, additions, deletions, fusion proteins and truncations in the polypeptide encoded
by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino
acid sequence from another, reference polypeptide. Generally, differences are limited so that the
sequences of the reference polypeptide and the variant are closely similar overall and, in many
regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one
or more substitutions, additions, deletions in any combination. A substituted or inserted amino
acid residue may or may not be one encoded by the genetic code. The present invention also includes
include variants of each of the polypeptides of the invention, that is polypeptides that vary from the
referents by conservative amino acid substitutions, whereby a residue is substituted by another with like
characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among
the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or
aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2
or 1 amino acids are substituted, deleted, or added in any combination. A variant of a polynucleotide
or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is
not known to occur naturally. Non-naturally occurring variants of polynucleotides and
polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other
recombinant methods known to skilled artisans.
EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Example 1  Strain selection, Library Production and Sequencing

The polynucleotide having a DNA sequence given in Table 1 [SEQ ID NO:1 and 3] was obtained from a library of clones of chromosomal DNA of *Streptococcus pneumoniae* in *E. coli*. The sequencing data from two or more clones containing overlapping *Streptococcus pneumoniae* DNAs was used to construct the contiguous DNA sequence in SEQ ID NO:1 or 3. Libraries may be prepared by routine methods, for example:

Methods 1 and 2 below.

Total cellular DNA is isolated from *Streptococcus pneumoniae* 0100993 according to standard procedures and size-fractionated by either of two methods.

Method 1

Total cellular DNA is mechanically sheared by passage through a needle in order to size-fractionate according to standard procedures. DNA fragments of up to 11kbp in size are rendered blunt by treatment with exonuclease and DNA polymerase, and EcoRI linkers added. Fragments are ligated into the vector Lambda ZapII that has been cut with EcoRI, the library packaged by standard procedures and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

Method 2

Total cellular DNA is partially hydrolyzed with a one or a combination of restriction enzymes appropriate to generate a series of fragments for cloning into library vectors (e.g., RsaI, Pali, Alul, Bshl235l), and such fragments are size-fractionated according to standard procedures. EcoRI linkers are ligated to the DNA and the fragments then ligated into the vector Lambda ZapII that have been cut with EcoRI, the library packaged by standard procedures, and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

Example 2  Functional oligomerization states of FtsZ

A $K_M$ of 200 $\mu$M and a $k_{cat}$ of 0.155 $sec^{-1}$ was determined for the GTPase activity of *E. coli* FtsZ using a real-time phosphate release assay. To correlate this activity with the polymerization state of FtsZ, several biophysical studies were undertaken. Analytical ultracentrifugation demonstrated that FtsZ can exist as monomer, dimer, or tetramer in the absence of nucleotide with $K_{1,2} = 1.5 \pm 0.96 \mu$M and $K_{2,4} = 10.0 \pm 0.28 \mu$M. The oligomeric state of FtsZ
in the presence of nucleotides and inhibitors was examined. Additionally, circular dichroism is being used to evaluate if structural changes are associated with the oligomeric and nucleotide-bound states of FtsZ. Refer to Figures 1-6.
What is claimed is:

1. An isolated multimeric FtsZ polypeptide selected from the group consisting of:
   (i) a multimeric FtsZ polypeptide comprising an isolated polypeptide comprising an amino acid sequence selected from the group having at least:
      (a) 75% identity;
      (b) 80% identity;
      (c) 90% identity; or
      (d) 95% identity
   to the amino acid sequence of SEQ ID NO:2 or 4 over the entire length of SEQ ID NO.2 or 4 respectively;
   (ii) a multimeric FtsZ polypeptide comprising isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 4 or
   (iii) a multimeric FtsZ polypeptide comprising isolated polypeptide which is the amino acid sequence of SEQ ID NO:2 or 4.

2. An antibody immunospecific for the polypeptide of claim 1.

3. A method for screening to identify compounds that activate or that inhibit the function of the polypeptide of claim 1 which comprises a method selected from the group consisting of:
   (a) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;
   (b) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presence of a labeled competitor;
   (c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;
   (d) mixing a candidate compound with a solution containing a polypeptide of claim 1, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard;
   (e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay, or
(f) (1) contacting a composition comprising the polypeptide with the compound to be screened under conditions to permit interaction between the compound and the polypeptide to assess the interaction of a compound, such interaction being associated with a second component capable of providing a detectable signal in response to the interaction of the polypeptide with the compound; and

(2) determining whether the compound interacts with and activates or inhibits an activity of the polypeptide by detecting the presence or absence of a signal generated from the interaction of the compound with the polypeptide.

4. An expression system comprising a polynucleotide capable of producing a polypeptide of claim 1 when said expression system is present in a compatible host cell.

5. A host cell comprising the expression system of claim 4 or a membrane thereof expressing a multimeric FtsZ polypeptide.

6. A process for producing a multimeric FtsZ polypeptide comprising the step of culturing a host cell of claim 8 under conditions sufficient for the production of said polypeptide.

7. A process for producing a host cell comprising a multimeric FtsZ polypeptide comprising the step of transforming or transfecting a cell with the expression system of claim 8 such the host cell, under appropriate culture conditions, produces a a multimeric FtsZ polypeptide polypeptide.

8. A host cell produced by the process of claim 7 or a membrane thereof expressing a multimeric FtsZ polypeptide comprising polypeptide.
Malachite Green Assay for GTP Hydrolysis

- Total consumption of GTP
- 100 µM GTP
- No GTP

[\text{E. coli ftsZ}] (\mu M)

\text{µmol PO}_4
at 36 °C,
\[ K_{1,2} = 1.49 \pm 0.96 \times 10^{-6} \text{ M} \]
\[ K_{2,4} = 1.00 \pm 0.28 \times 10^{-5} \text{ M} \]

at 2 \times 10^{-6} \text{ M FtsZ},

\[ [\text{monomer}] = 46\% \]
\[ [\text{dimer}] = 49\% \]
\[ [\text{tetramer}] = 5\% \]
Temperature Dependence of *E. coli* FtsZ K1,2 and K2,4
FtsZ, E. coli, 200 uM GDP

at 36 °C, 200 uM GDP

\[ K_{1,2} = 2.16 \pm 0.40 \times 10^{-4} \text{ M} \]

\[ K_{2,4} = 1.55 \pm 0.64 \times 10^{-6} \text{ M} \]
FtsZ, E coli, 200 uM gS-GTP

At 36 °C, 200 uM gS-GTP

K_{1,2} = 1.67 \pm 0.12 \times 10^{-3} M

K_{2,4} = 7.06 \pm 0.12 \times 10^{-5} M
SEQUENCE LISTING

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<120> FTS2 MULTIMERIC PROTEINS AND THEIR USES

<130> GM50046

<150> 60/091,680
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<213> Streptococcus pneumoniae

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20 25 30
His Gly Met Asn Asn Val Glu Phe Ile Ala Ile Asn Thr Asp Gly Gln
35 40 45
 Ala Leu Asn Leu Ser Lys Ala Glu Ser Lys Ile Gln Ile Gly Glu Lys
50 55 60
 Leu Thr Arg Gly Leu Gly Ala Gly Ala Asn Pro Glu Ile Gly Lys Lys
65 70 75 80
 Ala Ala Glu Gly Ser Arg Glu Gln Ile Glu Asp Ala Ile Gln Gly Ala
85 90 95
 Asp Met Val Phe Val Thr Ser Gly Met Gly Gly Thr Gly Thr Gly
100 105 110
 Ala Ala Pro Val Val Ala Lys Ile Ala Lys Glu Met Gly Ala Leu Thr
115 120 125
 Val Gly Val Val Thr Arg Pro Phe Ser Phe Glu Gly Arg Lys Arg Gln
130 135 140
 Thr Gln Ala Ala Ala Gly Val Glu Ala Met Lys Ala Ala Val Asp Thr
145 150 155 160
 Leu Ile Val Ile Pro Asn Asp Arg Leu Leu Asp Ile Val Asp Lys Ser
165 170 175
Thr Pro Met Met Glu Ala Phe Lys Glu Ala Asp Asn Val Leu Arg Glu
180 185 190
Gly Val Glu Gly Ile Ser Asp Leu Ile Ala Val Ser Gly Glu Val Asn
195 200 205
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210 215 220
Leu Met Gly Ile Gly Val Ser Ser Gly Glu Asn Arg Ala Val Glu Ala
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## INTERNATIONAL SEARCH REPORT

### A. CLASSIFICATION OF SUBJECT MATTER

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### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>WANG, X. et al. The FtsZ Protein of Bacillus subtilis is localized at the division site and has GTPase activity that is dependent upon FtsZ concentration. Molecular Microbiology. 1993, Vol. 9, No. 3, pages 435-442, entire document.</td>
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[X] Further documents are listed in the continuation of Box C. See patent family annex.

| * | Special categories of cited documents: |
| **T** | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| **A** | document defining the general state of the art which is not considered to be of particular relevance |
| **E** | earlier document published on or after the international filing date |
| **L** | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) |
| **O** | document referring to an oral disclosure, use, exhibition or other means |
| **P** | document published prior to the international filing date but later than the priority date claimed |

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
JOYCE BRIDERS
PARALEGAL SPECIALIST
CHEMICAL MATRIX

MANJUNATH RAO
Telephone No. (703) 308-0196
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**Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.:  
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.:  
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.:  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

   Please See Extra Sheet.

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   1

**Remark on Protest**

□ The additional search fees were accompanied by the applicant's protest.

□ No protest accompanied the payment of additional search fees.
BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim 1, drawn to an isolated multimeric polypeptide.

Group II, claim 2, drawn to an immunospecific antibody for polypeptide.

Group III, claim 3, drawn to a method for screening to identify compounds that activate or inactivate the polypeptide.

Group IV, claims 4-8, drawn to polynucleotide, expression vector and host cell.

The inventions listed as Groups I, II, III, IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The invention of Group I is a protein well known in the art. Identical protein has been isolated from a number of different bacteria and thus lacks novelty.

The invention of Group I is a product; this shares the technical feature of an enzyme, which groups II-IV do not share.

The invention of Group II is a product; this shares the technical feature of an antibody, which groups I, III and IV do not share.

The invention of Group III is a process which shares the special technical feature of chemical compounds that bind to the polypeptide and modulate its activity, which groups I, II and IV do not share.

The invention of Group IV is a product and a process of use; these share special technical feature of polynucleotides, which groups I and III do not share.