A statutory invention registration is not a patent. It has the defensive attributes of a patent but does not have the enforceable attributes of a patent. No article or advertisement or the like may use the term patent, or any term suggestive of a patent, when referring to a statutory invention registration. For more specific information on the rights associated with a statutory invention registration see 35 U.S.C. 157.
HindIII (29)

ampR

pJP-nv

pJAH142
(7356 bp)

lact

pT7

Ndel (2037)
Penicillin Binding Protein from Streptococcus Pneumoniae

This is a division of application Ser. No. 08/731,716, filed Oct. 17, 1996, now U.S. Pat. No. 5,789,202.

Background of the Invention

This invention relates to recombinant DNA technology. In particular, the invention pertains to the cloning of a gene, ppb-nv, encoding a novel high molecular weight penicillin binding protein (PBP), PBP-Nv, from Streptococcus pneumoniae and the use of said gene and its encoded protein in a screen for new inhibitors of bacterial cell wall biosynthesis.

The emergence of antibiotic resistance in common pathogenic bacterial species has justifiably alarmed the medical and research communities. The emergence and rapid spread of beta-lactam resistance in Streptococcus pneumoniae has been particularly problematic. This organism is responsible for many respiratory tract infections, and resistance to beta-lactam drugs has been attributed to a modification of one or more of the penicillin-binding proteins (PBPs). Furthermore, penicillin-resistant Streptococcus pneumoniae are frequently resistant to other commonly used antibiotics, such as erythromycin. These multi-drug resistant (MDR) organisms are a real threat to humans, particularly children and the elderly. Increasingly, the only drug that can be used to treat infections with MDR organisms is vancomycin, and there is considerable concern that the bacteria could develop resistance to vancomycin.

The PBPs are involved in bacterial cell wall synthesis. The cell wall comprises a peptidoglycan layer which provides mechanical rigidity for the bacterium. The peptidoglycan layer is composed of a sugar backbone (alternating residues of N-acetylmuramic acid and N-acetylmuramic acid) attached to a pentapeptide (also referred to as "stem peptide") containing D and L amino acid residues. In the formation of the mature peptidoglycan, a lipid-linked disaccharide-pentapeptide is translocated across the cytoplasmic membrane, exposing the pentapeptide sidechains to the cell surface. Transglycosylation of the sugar residues then leads to polymerization of the backbone sugar residues. Further stabilization of the nascent peptidoglycan occurs by a transpeptidation enzymatic reaction that crosslinks adjacent pentapeptide moieties. The high molecular weight PBPs catalyze these final steps in peptidoglycan synthesis. Without the crosslinking step the peptidoglycan structure is severely weakened and susceptible to degradation. Indeed, the crosslinking step constitutes the target of action for antibiotic compounds such as penicillin and other beta-lactam drugs.

When effective as antibiotic agents, beta-lactam drugs interact with PBPs to form an acyl-enzyme intermediate. This intermediate is resistant to hydrolysis. Mechanistically, beta-lactam drugs act as irreversible inhibitors. Resistance to beta-lactam drugs in Streptococcus pneumoniae arises through mutation events such that one or more low-affinity "mosaic" PBPs replace a wild-type PBP. The molecular basis of resistance has in a few cases been correlated with specific mutations within a PBP gene. The discovery of new antibacterial compounds against the transpeptidase domain of PBP-Nv or to an unexploited target (e.g., the transglycosylase domain) would be particularly useful against Streptococcus pneumoniae infections.

Summary of the Invention

The present invention is designed to meet the aforementioned need and provides, inter alia, isolated nucleic acid molecules that encode a novel PBP from Streptococcus pneumoniae. The invention also provides protein products encoded by the gene, in substantially purified form.

Having the cloned ppb-nv gene of Streptococcus pneumoniae enables the production of recombinant PBP-Nv protein and derivatives thereof for the implementation of assays and screens to identify new inhibitory compounds targeted at the peptidoglycan biosynthetic pathway.

In one embodiment the present invention relates to isolated gene ppb-nv that encodes novel Streptococcus pneumoniae PBP, PBP-Nv, said gene comprising the nucleotide sequence identified as SEQ ID NO. 1.

In another embodiment the present invention relates to a novel protein molecule, PBP-Nv, wherein said protein molecule comprises the sequence identified as SEQ ID NO. 2.

In another embodiment, the present invention relates to a soluble form of PBP-Nv (designated PBP-NvS) wherein PBP-NvS comprises amino acid residues 78 through 731, inclusive, of SEQ ID NO.2.

In a further embodiment the present invention relates to a ribonucleic acid molecule encoding PBP-Nv protein, said ribonucleic acid molecule comprising the sequence identified as SEQ ID NO. 3.

In yet another embodiment, the present invention relates to a recombinant DNA vector that incorporates the Streptococcus pneumoniae ppb-nv gene in operable linkage to gene expression sequences enabling said PBP gene to be transcribed and translated in a host cell.

In still another embodiment the present invention relates to homologous or heterologous host cells that have been transformed or transfected with a vector carrying the cloned ppb-nv gene from Streptococcus pneumoniae such that said gene is expressed in the host cell.

In a still further embodiment, the present invention relates to a method for identifying compounds that bind the Streptococcus pneumoniae PBP-Nv protein or fragment thereof.

Description of the Drawing

Figure. Plasmid JAH142, useful for high level expression of the Streptococcus pneumoniae ppb-nv gene of the present invention in the heterologous procaryotic host cell Escherichia coli.

Definitions

"PBP" refers generically to a penicillin binding protein.

"PBP-Nv" refers to the novel high molecular weight PBP from Streptococcus pneumoniae that is the subject of this invention and which is specified by SEQ ID NO.2.

"PBP-NvS" refers to a soluble form of PBP-Nv wherein the membrane spanning region and the cytoplasmic region of PBP-Nv have been removed, leaving amino acid residues 78 through 731 of SEQ ID NO.2.

"ppb-nv" refers to the Streptococcus pneumoniae genomic sequence encoding PBP-Nv.

"ppb-nvS" refers to a portion of ppb-nv that encodes PBP-Nv comprising nucleotide residues 232 through 2193 of SEQ ID NO.1.

The terms "cleavage" or "restriction" of DNA refers to the catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA (viz. sequence-specific endonucleases). The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements are used in the manner well known to one of ordinary skill in the art.
Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer or can readily be found in the literature.

The term “fusion protein” denotes a hybrid protein molecule not found in nature comprising a translational fusion or enzymatic fusion in which two or more different proteins or fragments thereof are covalently linked on a single polypeptide chain.

“Functional domain” refers to a region of a protein having one or more distinct biological functions, for example, enzymatic activity, transmembrane anchoring, DNA binding, etc. A functional domain comprises a sequence of amino acids, the length of which and the identity of amino acid residues therein, may or may not be critical to function.

The term “plasmid” refers to an extrachromosomal genetic element. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accordance with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

“Recombinant DNA cloning vector” as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been added.

The term “recombinant DNA expression vector” as used herein refers to any recombinant DNA cloning vector, for example a plasmid or phage, in which a promoter and other regulatory elements are present to enable transcription of the inserted DNA.

The term “vector” as used herein refers to a nucleic acid compound used for introducing exogenous DNA into host cells. A vector comprises a nucleotide sequence which may encode one or more protein molecules. Plasmids, cosmids, viruses, and bacteriophages, in the natural state or which have undergone recombinant engineering, are examples of commonly used vectors.

The terms “complementary” or “complementarity” as used herein refers to the capacity of purine and pyrimidine nucleotides to associate through hydrogen bonding in double stranded nucleic acid molecules. The following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil.

“Isoelectric nucleic acid compound” refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

A “primer” is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid molecule.

The term “promoter” refers to a DNA sequence which directs transcription of DNA to RNA.

A “probe” as used herein is a labeled nucleic acid compound that hybridizes with another nucleic acid compound.

The term “hybridization” as used herein refers to a process in which a single-stranded nucleic acid molecule joins with a complementary strand through nucleotide base pairing. “Selective hybridization” refers to hybridization under conditions of high stringency. The degree of hybridization depends upon, for example, the degree of complementarity, the stringency of hybridization, and the length of hybridizing strands.

The term “stringency” refers to hybridization conditions. High stringency conditions disfavor non-homologous base-pairing. Low stringency conditions have the opposite effect.

Stringency may be altered, for example, by temperature and salt concentration.

“Transglycosylation” refers to an enzymatic reaction catalyzed by a high molecular weight PBP in which the sugar residues of lipid-linked disaccharide pentapeptide molecules are polymerized during the formation of the peptidoglycan structure of the bacterial cell wall.

“Transpeptidation” refers to an enzymatic reaction catalyzed by a high molecular weight PBP in which the pentapeptide sidechains of lipid-linked disaccharide pentapeptide molecules are cross-linked during the formation of the peptidoglycan structure of the bacterial cell wall.

**Detailed Description**

The pbp-nv gene (SEQ ID NO.1) of the present invention encodes a novel high molecular weight PBP of Streptococcus pneumoniae (SEQ ID NO. 2). The pbp-nv gene disclosed herein comprises a DNA sequence of 2193 nucleotide base pairs (SEQ ID NO. 1). There are no intervening sequences. Those skilled in the art will recognize that owing to the degeneracy of the genetic code (i.e. 64 codons which encode 20 amino acids), numerous “silent” substitutions of nucleotide base pairs could be introduced into the sequence identified as SEQ ID NO. 1 without altering the identity of the encoded amino acid(s) or protein product. All such substitutions are intended to be within the scope of the invention.

The PBP-Nv protein defined by SEQ ID NO.2 comprises a membrane-bound protein having several functional domains. At the amino terminal end, amino acid residues from about 1 through 56 of SEQ ID NO.2 define a cytoplasmic domain. The middle portion of the molecule from about amino acid residues 57 through 77 of SEQ ID NO.2, comprises a trans-membrane region. At the carboxyl terminal end of the molecule, which extends into the extra-cellular environment, amino acid residues from about 78 through 731 of SEQ ID NO.2 comprise a functional domain, wherein resides the transpeptidation (viz. penicillin-binding) and transglycosylase activities.

The PBP-Nv protein may be modified by deletion of one or more functional domains. For example, the amino terminal domain and trans-membrane domains may be deleted without a loss of function at the carboxy terminal end (viz. binding of penicillin and transglycosylase activity). A deleted form of the PBP-Nv protein lacking the amino terminal and transmembrane regions results in a soluble form of the protein, designated PBP-NvS.

**Gene Isolation Procedures**

Those skilled in the art will recognize that the gene of the present invention may be obtained by a plurality of applicable genetic and recombinant DNA techniques including, for example, polymerase chain reaction (PCR) amplification, or de novo DNA synthesis. (See e.g., J. Sambrook et al. Molecular Cloning, 2d Ed. Chap. 14 (1989)).

Methods for constructing gene libraries in a suitable vector such as a plasmid or phage for propagation in prokaryotic or eucaryotic cells are well known to those skilled in the art. [See e.g. J. Sambrook et al. Supra]. Suitable cloning vectors are widely available.

Skilled artisans will recognize that the pbp-nv gene of Streptococcus pneumoniae comprising the present invention or fragment thereof could be isolated by PCR amplification of Streptococcus pneumoniae genomic DNA or cDNA using

The amplification reaction comprises genomic DNA, suitable enzymes, primers, and buffers, and is conveniently carried out in a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, Conn.). A positive result is determined by detecting an appropriately-sized DNA fragment following agarose gel electrophoresis.

Protein Production Methods

One embodiment of the present invention relates to the substantially purified protein or fragments thereof encoded by the gene disclosed herein (SEQ ID NO.1). Skilled artisans will recognize that the protein of the present invention can be synthesized by any number of different methods. The amino acid compounds of the invention can be made by chemical methods well known in the art, including solid phase peptide synthesis or recombinant methods. Both methods are described in U.S. Pat. No. 4,617,149, incorporated herein by reference.

The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area. See, e.g., H. Dugas and C. Penney, Bioorganic Chemistry (1981) Springer-Verlag, New York, 54–92. For example, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (Applied Biosystems, Foster City, CA) and synthesis cycles supplied by Applied Biosystems. Protected amino acids, such as t-butoxycarbonyl-protected amino acids, and other reagents are commercially available from many chemical supply houses.

The protein of the present invention can also be produced by recombinant DNA methods using the cloned gene of Streptococcus pneumoniae disclosed herein. Recombinant methods are preferred if a high yield is desired. Expression of said cloned gene can be carried out in a variety of suitable host cells well known to those skilled in the art. In a recombinant method, the pbp-nv or pbp-nv-gene is introduced into a host cell by any suitable means, well known to those skilled in the art. While chromosomal integration of the cloned PBP gene is within the scope of the present invention, it is preferred that the gene be cloned into a suitable extra-chromosomally maintained expression vector so that the coding region of the gene is operably linked to a constitutive or inducible promoter.

The basic steps in the recombinant production of PBP-Nv or PBP-Nv-gene of the present invention are:

a) constructing a natural, synthetic or semi-synthetic DNA encoding said PBP protein;

b) integrating said DNA into an expression vector in a manner suitable for expressing said PBP protein, as the natural protein product or as a fusion protein;

c) transforming or otherwise introducing said vector into an appropriate eucaryotic or prokaryotic host cell forming a recombinant host cell,

d) culturing said recombinant host cell in a manner enabling expression of said protein; and

e) recovering and substantially purifying said protein by any suitable means, well known to those skilled in the art.

Expressing Recombinant PBP Proteins in Procyotary and Eucaryotic Host Cells

In general, procaryotes are used for cloning DNA sequences and for constructing the vectors of the present invention. Procaryotes may also be employed in the production of a novel PBP protein of the present invention. For example, the Escherichia coli K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of E. coli, bacilli such as Bacillus subtilis, enterobacteriaceae such as Salmonella typhimurium or Serratia marcescens, various Pseudomonas species and other bacteria, such as Streptomyces, may also be employed as host cells in the cloning and expression of the recombinant proteins of this invention.

Promoter sequences suitable for driving the expression of genes in procaryotes include β-lactamase (e.g., vector pGXB2907, ATCC 39344, contains a replicon and β-lactamase gene), lactose systems (Chang et al., Nature (London), 281:544 (1979)), alkaline phosphatase, and the tryptophan (trp) promoter system (vector pATH11 (ATCC 37605) which is designed to facilitate expression of an open reading frame as a trpE fusion protein under the control of the trp promoter). Hybrid promoters such as the tac promoter (isolatable from plasmid pDR540, ATCC-37282) are also suitable. Still other promoters, such as that from bacteriophage T7, whose nucleotide sequences are generally known, enable one of skill in the art to ligate such promoter sequences to DNA encoding the proteins of the instant invention using linkers or adapters to supply any required restriction sites. Still other promoters are useful for gene expression in S. pneumoniae, for example the am pl promoter (J. P. Claverys et al., "Construction and evaluation of new drug-resistance cassettes for gene disruption mutagenesis in Streptococcus pneumoniae, using an am p test platform," Gene (1995) 123–128) Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

The proteins of this invention may be synthesized either by direct expression or as a fusion protein comprising the protein of interest as a translational fusion with another protein or peptide which may be removable by enzymatic or chemical cleavage. It is often observed in the production of certain peptides in recombinant systems that expression as a fusion protein prolongs the lifespan, increases the yield of the desired peptide, or provides a convenient means of purifying the protein. A variety of peptideases (e.g. enterokinase and thrombin) that cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (e.g. diaminopeptidase) of the peptide chain are known. Furthermore, particular chemicals (e.g. cyanogen bromide) will cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semi-synthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. See e.g., P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13, in Protein Purification: From Molecular Mechanisms to Large Scale Processes, American Chemical Society, Washington, D.C. (1990).

In addition to procaryotes, a variety of eucaryotic microorganisms such as yeast are suitable host cells. The yeast Saccharomyces cerevisiae is the most commonly used eucaryotic microorganism. A number of other yeasts such as Kluyveromyces lactis are also suitable. For expression in Saccharomyces, the plasmid YRp7 (ATCC-30053), for example, may be used. See, e.g., L. Stinchcomb, et al., Nature, 282:39 (1979); J. Kim, et al., Gene, 7:141 (1979); S. Tschemper et al., Gene, 10:157 (1980). Plasmid YRp7 contains the TRP1 gene which provides a selectable marker for use in a trp1 auxotrophic mutant.
Purification of Recombinantly-Produced PBP-Nv and PBP-Nv•

An expression vector carrying cloned pbp-Nv or pbp-Nv• of Streptococcus pneumoniae is transformed or transduced into a suitable host cell using standard methods. Cells that contain the vector are propagated under suitable conditions for expression of the encoded PBP. If the gene is under the control of an inducible promoter, suitable growth conditions would incorporate an appropriate inducer. Recombinant-produced PBP-Nv• or PEP-Nv protein may be purified from cellular extracts of transformed cells by any suitable means. Recombinant-produced PEP-Nv is expected to be partially localized in the host cell membrane. As such, PBP-Nv is recoverable from cell extracts and cell membranes by any suitable means, well known to those skilled in the art.

In a preferred process for protein purification the gene encoding the PEP of the present invention is modified at the 5' end to incorporate several histidine residues at the amino terminal end of the encoded protein product. The "histidine tag" enables a simplified protein purification method referred to as "immobilized metal ion affinity chromatography" (IMAC), essentially as described in U.S. Pat. No. 4,569,794, which thereby is incorporated by reference. The IMAC method enables rapid isolation of substantially pure protein starting from a crude cellular extract.

Other embodiments of the present invention comprise isolated nucleic acid sequences. As skilled artisans will recognize, owing to the degeneracy of the genetic code the amino acid compounds of the invention can be encoded by a multitude of different nucleic acid sequences. Because these alternative nucleic acid sequences would encode the same amino acid sequences, the present invention further comprises these alternate nucleic acid sequences.

The nucleic acid sequences encoding SEQ ID NO: 2, and disclosed subregions therein may be produced using synthetic methodology. The synthesis of nucleic acids is well known in the art. See, e.g., E. L. Brown, R. Belagaç, M. J. Ryan, and H. G. Khorana, Methods in Enzymology, 68:109–151 (1979). The DNA segments corresponding to the pbp-Nv or related gene sequence pbp-Nv• could be generated using a conventional DNA synthesizing apparatus, such as the Applied Biosystems Model 380A or 380B DNA synthesizers (Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, Calif. 94404) which employ phosphoramidite chemistry. Alternatively, phosphotriester chemistry may be employed to synthesize the nucleic acids of this invention. [See, e.g., M. J. Gait, ed., Oligonucleotide Synthesis. A Practical Approach, (1984).]

In an alternative and preferred methodology, namely PCR, the DNA sequence comprising a portion or all of SEQ ID NO: 3, can be generated from Streptococcus pneumoniae genomic DNA using suitable oligonucleotide primers complementary to SEQ ID NO: 1 or region therein, as described in U.S. Pat. No. 4,889,818, which hereby is incorporated by reference. Suitable protocols for performing the PCR are widely known and are disclosed in, for example, PCR Protocols: A Guide to Method and Applications, Ed. Michael A. Innis et al., Academic Press, Inc. (1990).

The ribonuclease acids of the present invention may be prepared using the polynucleotide synthetic methods discussed supra, or they may be prepared enzymatically using RNA polymerase to transcribe a suitable DNA template.

The most preferred systems for preparing the ribonuclease acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. These RNA polymerases are highly specific, requiring the insertion of bacteriophage-specific sequences at the 5' end of the template to be transcribed. See, J. Sambrook, et al., supra, at 18.82–18.84.

This invention also provides nucleic acids, RNA or DNA, that are complementary to SEQ ID NO: 1 or SEQ ID NO: 3.

The present invention also provides probes and primers useful for a variety of molecular biology techniques including, for example, hybridization screens of genomic or subgenomic libraries. A nucleic acid compound comprising SEQ ID NO: 1, SEQ ID NO: 3 or a complementary sequence thereof, or a fragment thereof, and which is at least 18 base pairs in length, and which will selectively hybridize to Streptococcus pneumoniae DNA or mRNA encoding the PBP of the present invention, is provided. Preferably, the 18 or more nucleotide bases are DNA. These probes and primers can be prepared by enzymatic methods well known to those skilled in the art (See e.g. Sambrook et al. supra). In a most preferred embodiment these probes and primers are synthesized using chemical means as described above.

Another aspect of the present invention relates to recombinant DNA cloning vectors and expression vectors comprising the nucleic acids of the present invention. Many of the vectors encompassed within this invention are described above. The preferred nucleic acid vectors are those that comprise DNA. The most preferred recombinant DNA vectors comprise the isolated DNA sequence, SEQ ID NO: 1. Plasmid JAH142 is an especially preferred DNA vector for expressing the soluble form of the PBP of this invention in E. coli.

The skilled artisan understands that choosing the most appropriate cloning vector or expression vector depends upon a number of factors including the availability of restriction enzymes, the type of host cell into which the vector is to be transfected or transformed, the purpose of the transfection or transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable or selectable markers (e.g., antibiotic resistance and metabolic markers of one type and another), and the number of copies of the gene to be present in the host cell.

Vectors suitable to carry the nucleic acids of the present invention comprise RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. The most preferred vectors are plasmids.

When preparing an expression vector the skilled artisan understands that there are many variables to be considered, for example, whether to use a constitutive or inducible promoter. Inducible promoters are preferred because they enable high level, regulatable expression of an operably linked gene. The skilled artisan will recognize a number of inducible promoters that respond to a variety of inducers, for example, carbon source, metal ions, heat, and others. The practitioner also understands that the amount of nucleic acid or protein to be produced dictates, in part, the selection of the expression system. The addition of certain nucleotide sequences is useful for directing the localization of a recombinant protein. For example, a sequence encoding a signal peptide preceding the coding region of a gene, is useful for directing the extra-cellular export of a resulting polypeptide.

Host cells harboring the nucleic acids disclosed herein are also provided by the present invention. A preferred host is E. coli that has been transfected or transformed with a vector that comprises a nucleic acid of the present invention.

The present invention also provides a method for constructing a recombinant host cell capable of expressing SEQ
ID NO:2, or the soluble form thereof, said method comprising transforming or otherwise introducing into a host cell a recombinant DNA vector that comprises an isolated DNA sequence which encodes SEQ ID NO:2 or fragment thereof. The preferred host cell is any strain of *E. coli* that can accommodate high level expression of an exogenously introduced gene. Preferred vectors for expression are those which comprise SEQ ID NO:1. An especially preferred expression vector for use in *E. coli* is pAH142, which comprises nucleotide residues 232 through 2193 of SEQ ID NO:1. (See Figure). Transformed host cells may be cultured under conditions well known to skilled artisans such that a recombinant protein is expressed, thereby producing in the recombinant host cell the PBP of the instant invention.

For the purpose of identifying or developing new antibiotic compounds it is useful to determine compounds that bind to PBPs and/or inhibit the transpeptidase or transglycosylase activity. The instant invention provides a screen for identifying compounds that bind PBP-Nv and/or PBP-Nv5 or fragment thereof, said screen comprising the steps of:

a) preparing and substantially purifying a recombinant PBP of the invention;

b) exposing said PBP to a test compound; and

c) monitoring, by any suitable means, the binding by said compound to said PBP, or inhibition of enzymatic activity of said PBP by said compound.

The substrate for a transglycosylase assay can be made according to art-recognized methods (See e.g. DiBerardino et al. FEBS Letters, 392, 184-88 (1996). For example, the lipid precursor substrate can be prepared from *Streptococcus pneumoniae* membranes, or from the membranes of any other suitable bacteria, UDP-Mur-Nac-pentapeptide, and UDP-N-acetyl-[14C]glucosamine (Amersham, Buckinghamshire, UK). Transglycosylase activity is measured by the production of the peptidoglycan polymerization product essentially by mixing the substrate with a source of PBP and monitoring the amount of [14C]-label in the peptidoglycan.

The above-disclosed screening system could also be used to identify compounds that inhibit the transpeptidase activity of PBP-Nv or PBP-Nv5. In a preferred embodiment of this aspect of the invention compounds are tested for their ability to competitively inhibit the binding of labeled penicillin to PBP-Nv or PBP-Nv5.

The screening system described above provides a means to determine compounds that interact with the PBPs of the present invention and which may interfere with peptidoglycan biosynthesis. This screening method may be adapted to automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system, allowing for efficient high-volume screening for potential inhibitory agents.

The following examples more fully describe the present invention. Those skilled in the art will recognize that the particular reagents, equipment, and procedures described below are merely illustrative and are not intended to limit the present invention in any manner.

**EXAMPLE 1**

**Construction of a DNA Vector for Expressing *Streptococcus pneumoniae* pbp-nv Gene in a Heterologous Host**

Plasmid JAH142 (See Figure) is an approximately 7300 base pair expression vector suitable for expressing a modified pbp-nv in prokaryotic host *E. coli*. This plasmid contains an origin of replication (Ori), an ampicillin resistance gene (Amp), useful for selecting cells that have incorporated the vector following a transformation procedure, and further comprises the T7 promoter in operable linkage to the coding region of said PBP gene. Parent plasmid pET11A (obtained from Novogen, Madison, Wis.) was linearized with digestion with endonucleases Ndel and HindIII. Linearized pET11A was ligated to a DNA fragment bearing Ndel and HindIII sticky ends, comprising a modified pbp-nv5 gene. The pbp-nv5 gene ligated into pJAH142 was modified at the 5′ end in order to simplify purification of the encoded protein product. For this purpose, an oligonucleotide encoding 8 histidine residues and a factor Xa cleavage site, Ile-Glu-Gly-Arg, was inserted at the 3′ end of an ATG start codon and at the 5′ end of residue 232 of SEQ ID NO:1. Placement of the histidine residues at the amino terminus of the encoded protein enables the IMAC protein purification procedure (See Example 4). An additional modification of the pbp-nv5 gene ligated into pJAH142 comprises two silent base pair substitutions, one at nucleotide residue 270 of SEQ ID NO:1, at which a “G” replaces an “A”, and the other at nucleotide residue 777 of SEQ ID NO:1, at which a “C” replaces a “T.” These substitutions do not alter the encoded amino acid sequence.

**EXAMPLE 2**

**Construction of a DNA Vector for Expression of pbp-nv in a Heterologous Host**

The plasmid construction method outlined in Example 1 is followed to construct a vector for expressing PBP-Nv in a heterologous host such as *E. coli*. A “his-tag” oligonucleotide comprising the same structure and sequence disclosed in Example 1 is inserted at the 3′ end of the ATG initiation codon at nucleotide position 3 of SEQ ID NO:1.

**EXAMPLE 3**

**Expression of *Streptococcus pneumoniae* pbp-nvS Gene in *Echerichia coli***

Expression plasmid JAH142 was transformed into *E. coli* BL21 (DE3) (F*ompT*lon*js* Dr− mve−) using standard methods (See e.g. Sambrook et al. Supra). Transformants, chosen at random were tested for the presence of pJAH142 by agarose gel electrophoresis using quick plasmid preparations. Id. Transformants were grown overnight at 37°C in LB medium supplemented with 100 μg/ml ampicillin. The overnight culture was diluted into fresh LB medium and allowed to grow to an O.D.600 of 0.4 to 0.6. At that point, expression of the vector-bound pbp-nvS gene was induced by adding 0.4 mM IPTG for a period of 3 hours. The induced-culture was then pelleted by centrifugation in preparation for protein purification (See Example 4).

**EXAMPLE 4**

**Purification of PBP-Nv5**

The recombinant cell pellet, isolated as described in the last step of Example 3, was resuspended in 60 ml of 20 mM potassium phosphate, pH 7.5. The cells were disrupted by passage through a French press, producing a cell extract that was centrifuged at 150,000g for 1 hour. The supernatant was loaded onto a 200 ml bed-volume XK50 source-Q column (Pharmacia) equilibrated in 20 mM potassium phosphate, pH 7.5 (buffer A). The column was washed with 500 ml buffer A and eluted with a linear gradient of 0 to 1M KCl in buffer A. Fractions were pooled, tested for penicillin...
binding activity, and the fractions showing binding activity were dialyzed overnight at 4°C against 20 mM potassium phosphate, pH 7 (buffer B). The dialyzed protein sample was applied to a XK26 hydroxyapatite column (60 ml bed volume) equilibrated in buffer B. After washing the column with buffer B, samples were eluted with a linear gradient of 20 mM to 700 mM potassium phosphate, pH 7. Fractions exhibiting penicillin binding activity were pooled and dialyzed against 20 mM Tris, pH 8.

The PBP-Nv protein contained in the pooled fractions was purified further by immobilized metal ion affinity chromatography (IMAC), essentially as described in U.S. Pat. No. 4,569,794, the entire contents of which is hereby incorporated by reference. Briefly, the IMAC procedure involved adding to the protein sample the following components at the indicated final concentrations: 0.5M NaCl, 5 mM imidazole. The sample was loaded onto a Chelating Sepharose Fast Flow column (Pharmacia, 10 ml bed volume) and the column washed twice with 35 ml each of 20 mM Tris, pH 8, 0.5M NaCl and 5 mM imidazole; 20 mM Tris, pH 8, 0.5M NaCl and 60 mM imidazole. The bound protein was eluted from the column with 20 mM Tris, pH 8, 0.5M NaCl, 1M imidazole. Six fractions of 3 ml each were collected and tested for penicillin binding activity (See Example 5).

EXAMPLE 5
Penicillin binds Streptococcus pneumoniae PBP-Nv

Protein fractions isolated from the IMAC column, as in Example 4, are tested for penicillin binding as follows. About 10 µl of the protein sample eluted from the column is mixed with 125I-labeled penicillin V at a final concentration of 48 µg/ml. Labeled penicillin is prepared as described in Blasczak et al. “Radiiododestannylation. Convenient synthesis of a stable penicillin derivative for rapid penicillin binding protein assay,” J. Labelled Compd. Radiopharm. 27, 401-06 (1989). About 3 µl of a 1 µg/µl solution of sodium clavulanate is added to prevent degradation of the labeling reagent. The mixture is incubated at 35°C for 15 minutes. Reactions are terminated by the addition of one-half volume SDS with boiling for 2 minutes. Aliquots of each mixture are fractionated by polyacrylamide gel electrophoresis, and radiolabeled bands are detected by exposure to X-ray film.

This method demonstrates a major labeled band at about 81 kilodalton (the predicted size based on amino acid sequence disclosed in SEQ ID NO.2).

EXAMPLE 6

Determination of PBP-Nv Transglycosylase activity

Radiolabeled lipid precursor for use as substrate is prepared as described in H. Hara and H. Suzuki FEBS Lett. 168, 155-60 (1984). Peptidoglycan synthesis activities are determined in 50 µl reactions containing 50 mM PIPES, pH 6.1, 10 mM MgCl2, 0.2 mM DTT, 1 mM ATP, 26% DMSO, PBP-Nv or PBP-Nvs sample and 14C-labelled lipid precursor. The reaction is incubated for 30 minutes at room temperature and filtered through hydrophilic Durapore PVDF membranes (0.65 Jm; Millipore, Bedford, Mass.). Under these conditions the synthesized peptidoglycan is retained while the unincorporated labeled substrate is washed through using 0.4 M ammonium acetate in methanol. The filter bound radioactivity is determined by scintillation counting.
CUT ACT GAT CGA AAA AAA TTA GCC CAA GTA GCT CGC ATT CGA AAA TTC
Arg Ser Asp Arg Lys Leu Ala Gin Val Gly Pro Ile Arg Lys Phe
35 40
TGTC CCT CAT TAT CAT CTA ACA AAG ATT ATC CTT ATA GTA GCT TGC AGT
Trp Arg Arg Tyr His Thr Leu Thr Lys Ile Ile Leu Ile Leu Gly Leu Ser Thr
59 70 75 90
GCA GUC TGG CTA GTT GGA ATC YAT TGG TTT GCT GTA GCC AAG TCG ACC
Ala Gly Leu Val Val Gly Ile Tyr Phe Ala Val Ala Lys Ser Thr
65 70 80
AAT TGC AAT GAT TGG CAA AAT GCC TGG AAA ACT CGG ACT ATT TTT
Asn Val Asn Asp Leu Gin Asn Ala Leu Lys Thr Arg Thr Leu Ile Phe
85 90 95
GAC CTC GAA GAA AAA GAG GCT GTG GCC TGG TCT GCT GAA AAG GUA ACC
Asp Arg Glu Glu Glu Ala Gly Ala Leu Ser Gly Gin Gly Lys Thr
100 105 110
TAT GCT GAG CGT ACT GAC AGT AAA AAC TGG CAG AAT GCT GTC ATT
Tyr Val Glu Leu Thr Asp Ile Ser Asn Leu Asn Ala Val Ile
115 120
GCC ACA GAA GAC CTT TCT TTC TAT AAA AAT GAG GAC ATT ACG TAT GAC
Ala Thr Glu Asp Arg Ser Phe Tyr Lys Asn Asp Gly Ile Asn Tyr Gly
130 135 140
CUT TCT TCC TGT GCT ATT GTC ACT GCT GAA GCT TCA GGT GGT GCC TCT
Arg Phe Phe Leu Ala Val Thr Val Thr Arg Ser Gly Ser Gly Ser
145 150 155 160
ACC ATT ACC CAA CAG CTC GCT GAA AAA GCC TAT TTA TCG CAG CAT CAA
Thr Ile Thr Gin Gin Leu Ala Asn Ala Tyr Leu Ser Gin Asp Gin
165 170 175
ACT GGT GAG AGA AAA GGG AAA GAA TCT TTC TCT GCT GCA GTA TAA GCG
Thr Val Glu Arg Lys Ala Gin Gin Leu Gin Ser Leu Ser Leu Ser Arg Ser
180 185 190
AAA AAA TAT AGT AAG GAG CAA ATT CTA ACC ATG TAC CCT AAC GCC GCT
Lys Lys Tyr Ser Lys Glu Gin Ile Leu Thr Met Tyr Leu Asn Ala
195 200 205
TAT TTT GGA ATT GGT GTG GTA GAA GAT GCC AGT AAG AAG ATT TAC
Tyr Phe Gly Asn Gin Val Thr Gly Val Gin Asp Ala Ser Lys Thr Tyr
210 215 220
TTT GGA GTT TCT GCA TCA GAA TGG AGT CTG GAT CAA GCC GCT GCT CTG
Phe Gly Val Ser Ala Gin Val Ser Leu Gin Gin Asp Gin A Asp Gin
225 230 235 240
GCA GGG CGG CTG ATG AAG CGG CGG CTG AAT CCC TGG AAT TCG GTA
Ala Gly Met Leu Lys Gly Pro Glu Leu Tyr Asn Pro Leu Asn Ser Val
245 250 255
GAA GAT TCT ACT AAT CGG GCC GAT ACT GTC TGG CAG AAT GTT GCA
Glu Asp Ser Thr Asn Arg Asp Thr Val Leu Gin Asn Met Val Ala
260 265 270
GCA GAA TAT ATT GAT AAA AAC CAA GAA ACC GAA GCT GAA GTT GAT
Ala Gly Tyr Ile Asp Lys Asn Gin Glu Thr Glu Ala Ala Glu Val Asp
275 280 285
ATG ACT TCG CAA TGG CAC GAT AAG TAT GAA GGA AAA ATC TCA GAT TAC
Met Thr Ser Gin Leu His Lys Tyr Glu Gly Ile Ser Asp Tyr
290 295 300
CTT GAC TCC TCT TAT TTT GAC GTG GTC ATT GAT GTA GCC AAT GCC TAC
Arg Tyr Pro Ser Tyr Phe Asp Ala Val Val Ala Val Ser Lys
305 310 315 320
TAT ATT CTA ACA GAG CAA GAG ATT GTC AAT AAT GCC TAC GCC ATT TAC
Tyr Asn Leu Thr Glu Glu Gin Ile Val Asn Arg Asp Lys Thr Arg Ile Tyr
325 330 335
ACA GAG CTG GAC CAA AAC TAC CAA GCA AAT ATG CAG ATT GTC TAT GAA
Thr Glu Leu Asp Gin Tyr Gin Ala Asn Met Gin Ile Val Tyr Glu
340 345 350
AAC ACA TCG CTA TTT CGG AGG GCA GAG GAT GUA AGC TTT CCT CAA TCA
355 360 365

GGA ACT GTA GCT CTC GAA CCG AAA ACA GGG GGA GTT GGT GTA GTG
370 375 380

GTT CAA GTT GAC CAT GAT AAA ACT GAA TTC CCG AAT TAC AAC TAT
385 390 395 400

GCA ACC CAA TCA AAG CTT AGT CCT GCT TCT ACA ATT AAG CTT TTA GTT
405 410 415

GAT TAT ACA CCA GCA GTT GAA GCA GCC TGG GCT TGG AAT AAG CAG TGG
420 425 430

GAT AAG CAT ACC ATG CAG TAT GAT GCT AAG GTT GAT AAG TAT GCA
435 440 445

GGG ATT AAA ACA AGT GCA GTA CCT ATG TAT CAA TCC TGG GCA GAA
450 455 460

TCG CTT AAT CTA CCT GCT GTT GCC ACT GTT AAT GTG GGT GTT GAC
465 470 475 480

AAG GCT TTT GCA GCA GCC AAA TCC GCA CTC AAT GCG GAA AAG TGC
485 490 495

GAC CTT GTT CCT GAT GCC TGG GCA AGC GGT GTT GAA ACC AAC CCT
500 505 510

ATT GAG GCT TCA GAA TAC GTT GCC TTT GCA AMY GAA GTT TTA AGT
515 520 525

CCT GAA GCT CAT TTT ATT AGT AAG ATT GAA ATT GCT AGT GCA CAA GTT
530 535 540

ATT GCC AGT CAT AAA AAT TCA CAA AAA CGG GTG ATT GAT AAG TCT GTA
545 550 555 560

GCT GAC AAG ACC AGT ATG ATG TGG GGG ACT TTC ACC AAC GGT ACC
565 570 575

GCT ATT AGT TCA TCG CCT GCA GAC TAT GTC ATG GCA GGG AAA ACT GGA
580 585 590

ACA ACT GAA GCA GTT TTC CAG CAG TAC ACA ACT GAC CAG TGG GTA
595 600 605

ATT CTT TAT ACT CGG GAT GTA GTG ATT AGC CAC TGG CCT GCC TTT CGG
610 615 620

ACC ACT GAT GAA AAT CAC TAT CTA GCT GCC TCT ACT TCA AAC GGT GCA
625 630 635 640

GCT CAT GTG TTT AGA AAC ATT GCC AAT ACT ATT TTA CCT TAT ACG CCA
645 650 655

GGA ACT ACC TTT ACG GTT GAA ATT GCT TAT AAG CAA AAT GGA ATT GCA
2016

Gly Ser Thr Phe Thr Val Glu Ala Asn Ala Tyr Lys Gln Asn Gly Ile Ala
CCA GCC AAT ACA AAA AGA CAA GTA CAA ACC AAT GAT AAT AGC CAG ACA Pro Ala Asn Thr Lys Arg Glu Val Glu Thr Asn Asp Asn Ser Glu Thr 675 690 695 2064
GAT GAT AAT TGG TCT GAT ATT CCA GGG CCT GCU CAA AGT CTA GAT Asp Asp Asn Leu Ser Asp Ile Arg Gly Arg Ala Gln Ser Leu Val Asp 690 695 700 2112
GAG GCC GCG GCT ATC TCA GAT GCG AGG ATT AAG GAA AAG GCT CAA Glu Ala Ser Arg Ala Ile Ser Asp Ala Lys Ile Lys Glu Ala Gln 705 710 715 720 2160
ACA ATA TGG GAT TCG ATA GTC AAT TTT GGC Thr Ile Trp Asp Ser Ile Val Asn Leu Phe Arg 725 730 2193

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Leu Asp Lys Leu Phe Glu Lys Phe Leu Ser Leu Phe Lys Lys 1 5 10 15
Glu Thr Ser Glu Leu Glu Ser Asp Ser Thr Ile Leu Arg Arg Ser 20 25 30
Arg Ser Asp Arg Lys Leu Ala Gln Val Gly Pro Ile Arg Lys Phe 35 40 45
Trp Arg Arg Tyr His Leu Thr Lys Ile Ile Leu Leu Gly Leu Ser 50 55 60
Ala Gly Leu Leu Val Gly Ile Tyr Leu Phe Ala Val Ala Lys Ser Thr 65 70 75 80
Asn Val Asn Asp Leu Gin Asn Ala Leu Lys Thr Arg Thr Leu Ile Phe 85 90 95
Asp Arg Glu Glu Lys Ala Gly Ala Leu Ser Gly Glu Gly Lys Gly Thr 100 105 110
Tyr Val Glu Leu Thr Asp Ile Ser Lys Asn Leu Gin Asn Ala Val Ile 115 120 125
Ala Thr Glu Asp Arg Ser Phe Tyr Lys Asn Gly Ile Asn Tyr Gly 130 135 140
Arg Phe Phe Leu Ala Ile Val Thr Ala Gly Arg Ser Gly Gly Gly Ser 145 150 155 160
Thr Ile Thr Gin Gin Leu Ala Lys Asn Ala Tyr Leu Ser Gin Asp Gin 165 170 175
Thr Val Glu Arg Lys Ala Lys Glu Phe Phe Leu Ala Leu Glu Leu Ser 180 185 190
Lys Lys Tyr Ser Lys Gly Gin Ile Leu Thr Met Tyr Leu Asn Asn Ala 195 200 205
Tyr Phe Gly Asn Gly Val Trp Gly Val Glu Asp Ala Ser Lys Lys Tyr 210 215 220
Phe Gly Val Ser Ala Ser Glu Val Ser Leu Asp Gin Ala Ala Thr Leu 225 230 235 240
Ala Gly Met Leu Lys Gly Pro Glu Leu Tyr Asn Pro Leu Asn Ser Val 245 250 255
Glu Asp Ser Thr Asn Arg Arg Asp Thr Val Leu Gin Asn Met Val Ala
Ala Gly Tyr Ile Asp Lys Asn Gin Glu Thr Glu Ala Ala Glu Val Asp
Met Thr Ser Gin Leu His Asp Lys Tyr Glu Gly Lys Ile Ser Asp Tyr
Arg Tyr Pro Ser Tyr Phe Asp Ala Val Val Asn Glu Ala Val Ser Lys
Tyr Asn Leu Thr Glu Glu Ile Val Asn Asn Gly Tyr Arg Ile Tyr
Thr Glu Leu Asp Gin Asn Tyr Gin Ala Asn Met Gin Ile Val Tyr Glu
Asn Thr Ser Leu Phe Pro Arg Ala Glu Asp Gly Thr Phe Ala Gin Ser
Gly Ser Val Ala Leu Glu Pro Lys Thr Gly Val Arg Gly Val Val
Gly Gin Val Ala Asp Asn Asp Lys Thr Gly Phe Arg Asn Phe Asn Tyr
 Ala Thr Gin Ser Lys Arg Ser Pro Gly Ser Thr Ile Lys Pro Leu Val
Val Tyr Thr Pro Ala Val Glu Ala Gly Trp Ala Leu Asn Lys Gin Leu
Asp Asn His Thr Met Gin Tyr Asp Ser Tyr Lys Val Asp Asn Tyr Ala
 Gly Ile Lys Thr Ser Arg Glu Val Pro Met Tyr Gin Ser Leu Ala Glu
 Ser Leu Asn Leu Pro Ala Val Ala Thr Val Asp Leu Gly Val Asp
 Lys Ala Phe Glu Ala Gly Lys Phe Gly Leu Asn Met Glu Lys Val
Asp Arg Val Leu Gly Val Ala Leu Gly Ser Gly Val Glu Thr Asn Pro
Leu Gin Met Ala Gin Ala Tyr Ala Ala Phe Ala Asn Glu Gly Leu Met
Pro Glu Ala His Phe Ile Ser Arg Ile Glu Aan Ala Ala Ser Gly Gin Val
Ile Ala Ser His Lys Asn Ser Gin Lys Arg Val Ile Asp Lys Ser Val
 Ala Asp Lys Met Thr Ser Met Met Leu Gly Thr Phe Thr Asn Gly Thr
 Gly Ile Ser Ser Ser Pro Ala Asp Tyr Val Met Ala Gly Lys Thr Gly
 Thr Thr Glu Ala Val Phe Asn Pro Glu Tyr Thr Ser Asp Gin Trp Val
Ile Gly Tyr Thr Pro Asp Val Val Ile Ser His Trp Leu Gly Phe Pro
Thr Thr Asp Glu Asn His Tyr Leu Ala Gly Ser Thr Ser Asn Gly Ala
Ala His Val Phe Arg Asn Ile Ala Asn Thr Ile Leu Pro Tyr Thr Pro
Gly Ser Thr Phe Thr Val Glu Asn Ala Tyr Lys Gin Asn Gly Ile Ala
 Pro Ala Asn Thr Lys Arg Gin Val Glu Thr Aan Asp Asn Ser Gin Thr
Asp Asp Asn Leu Ser Asp Ile Arg Gly Arg Ala Gln Ser Leu Val Asp
693 695 700
Glu Ala Ser Arg Ala Ile Ser Asp Ala Lys Ile Lys Glu Lys Ala Gln
705 710 715 720
Thr Ile Trp Asp Ser Ile Val Asn Leu Phe Arg
725 730

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2193 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AUGAAANUAAG AUAUAAUUAAG UAGAAAUUU AUAUCUCUUU UUAAAAGAA AAACAGUGAA 60
CUACAGACGU CUGAUCUCUA UAAUACAGU CGCUUACUUA GUGUGCAA AAAAAUAGCC 120
CAAGUACCU CGAAGCGAAA AUAUCAGCGU CUGAUCACUC UAAACAAAGU UAUCUUAUA 180
CUACUUUGUA GUUGACGUUC GCUAGUGA UAUCUAAUU UUCUCUCAAC ACAGUCACCC 240
AAAGACACUA AUUGACAAA AUCUCUUAAA ACUGAGAAGC UUAUUAUUGA CCGUGAAGAA 300
AAACAGCUG ACUGCCUGUC UGCGUAAGGA GAAACCAGUG UUGACUCAGC UGACACAGU 360
AAACACUGG AAGCGCCUGU AUAUGCCACA GAAGACCGU UCUUCAUAUA AAAGACCCGG 420
AUUAACAUUG CCGUUCUUU CUUGGCUUUA UUAACUCUGG GACGUGCAAG UAGUGGCUCU 480
ACCAUAACCC AACAAGCGGC UAAAGGCCG UUAUUAUGG AAGAGAAGAA CGUUGGAGA 540
AAAGCAGAAC AUAUUKUUGCU UGCUUAAGAA UUAACGAAA AUAUCAGGU GAAGCAAAU 600
CUACUAAGGU ACCUUAACCA CCUCUUUUGA GGAAGGUGG UUGGCGGUGA AGAAAGACCG 660
AUGAAGAAAU AUCUUGACAGU UUUCGCAUCA GAAGUGACUG GUAUCAAGG UCGGACUUG 720
GCAGCGGCUG UCAAAGGCGG GGACUGUAU UAUUCCUGAA AUUCUGUAGA AGAUCAGCUC 780
AUGCGAAGCG UACUGUGAUG UCAGAAAUGG UGUCGAGAA GAAGAAGCGG UAAAACAAAGC 840
GAAACCGAA CGUGCCAGAGU UGAGAACGGU UCUGCAAGUG GCAGAAUAAG UGAAAGAAAA 900
AUCUGCAAGU ACCUUGAACU CUCUUAUUGU UACUGGUUGGC UUAUAGAACU UGUCUCAAAG 960
UAUAACUAAG CAAAGAGAGA GAUGUCAAGA AAGCGACUCC GCAUUAACAC AGAUCUGGAC 1020
CAACAAUU GAAACAAUAAG ACGCAGUUGU UAGAAACAAC CAUGCUUAAU UCCAGGCGCA 1080
GAGGAGUGAA CGUGUGCUCA AUAGAAGAAGU GUAACUCUCG AACAGAAAAC AGCGGAGGCUU 1140
GUUGAGUGUG UGCGUGCAAGU UCCUGCAGAU UAAAACAGU GAUGCCGGAU UAACCAUAU 1200
GCACAACCAU CAAAGGCGUG UCCGGGCUUC ACAAUAAGC CUUUAGGUGU AAUAUACCCA 1260
GCAGUGUGAG GCACGCGGCU UUGAUAUAGC CAGUUGGAAU ACCAUAAGAU CGAUGUGA 1320
AGCAACAGUU UAGCGGCGUA UGAAGAGAC AAAAAAGGGA GAGAGGCUCC UAAUAACUA 1380
UCUCAGCGAA AAGCGGCUAA UCUACUGGUU GUUGCCAGUG UUAAUGAAGU UGUGUGUAC 1440
AAAGCUUUGG AGCGAGCGAA AAAAAUGGAGA CUUACAAUG AAAAGGGUGA CCUGUGUGCUU 1500
GGUGUCGCCU UGGAGAAGCG UGUGAAACCC ACCCUCUUC AAAAAUGCUA AGCAUACGCU 1560
We claim:

1. An isolated Penicillin Binding Protein Nv (PBP-Nv) from *Streptococcus pneumoniae* having the amino acid sequence which is a SEQ ID NO:2.

2. An isolated Penicillin Binding Protein Nv (PBP-Nv) from *Streptococcus pneumoniae* having the amino acid sequence which is defined by residue 78 through 731 of SEQ ID NO:2.

3. A method for identifying compounds that bind a *Streptococcus pneumoniae* PBP-Nv, comprising the steps of:

   a) admixing:
   i) a substantially pure PBP wherein said PBP is PBP-Nv or PBP-Nv'; and
   ii) a suitable test compound;

   b) measuring by any suitable means binding by said compound to said PBP.

4. A method for identifying compounds that inhibit the transglycosylase activity of a *Streptococcus pneumoniae* PBP-Nv, comprising the steps of:

   a) admixing:
     i) a substantially pure PBP wherein said PBP is PBP-Nv or PBP-Nv'; and
     ii) a suitable substrate and test compound;

   b) measuring by any suitable means inhibition of said transglycosylase activity.