(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 May 2006 (11.05.2006)

PCT

(10) International Publication Number
WO 2006/048753 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/IB2005/003324

(22) International Filing Date: 24 October 2005 (24.10.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/625,533 5 November 2004 (05.11.2004) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/048753 A2

(54) Title: ANTI-BACTERIAL VACCINE COMPOSITIONS

(57) Abstract: The present invention relates generally to the identification of genes responsible for virulence of streptococci, thereby allowing for production of novel attenuated mutant strains useful in vaccines and identification of new anti-bacterial agents that target the virulence genes and their products.

ANTI-BACTERIAL VACCINE COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates generally to the identification of genes responsible for virulence of streptococci, thereby allowing for production of novel attenuated mutant strains useful in vaccines and identification of new anti-bacterial agents that target the virulence genes and their products.

BACKGROUND OF THE INVENTION

The genus *Streptococcus* encompasses several significant pathogens that infect a wide variety of animals. There are a large variety of *Streptococcus* species, many of which cause infections in animals and man. Streptococci are gram positive cocci occurring in pairs and chains. The main groups of streptococcal diseases include upper respiratory infections with lymphadenitis (horses, swine, cats, guinea pigs, humans) neonatal respiratory and septicemic infections (horses, swine, dogs, humans), secondary pneumonias and complications (horses, non-human primates, small carnivores, humans) and pyrogenic infections unrelated to the respiratory tract (human genitourinary tract infections, bovine mastitis).

Little is known about the mechanism of pathogenesis of diseases caused by streptococci. Various cellular components, such as muramidase-released protein (MRP), extracellular factor (EF), and cell-membrane associated proteins, fimbriae, haemagglutinins, and haemolysin, have been suggested as virulence factors. However, the precise role of these protein components in the pathogenesis of the various diseases remains unclear. There is a pressing need therefore to identify additional virulence genes in the streptococci. In addition, there remains a need for safe and effective vaccines for treating and preventing diseases caused by streptococci.

Information Disclosure - Patent Documents

US20020055168 A1 Streptococcus suis vaccines and diagnostic tests

WO2003046183 A2 Streptococcus suis avirulent vaccine and uses in antibiotic design

US20030072769 A1 Virulence genes, proteins, and their use

US20040009192 A1 Virulence of streptococci

WO2002061070 A2 Environmentally regulated genes, involved in the virulence of Streptococcus suis.

SUMMARY OF THE INVENTION

In general, the present invention provides materials and methods for production and use of attenutated gram positive bacteria. In one aspect the invention comprises attenuated species in the streptococcal family of bacteria.

The attenuated bacteria of the invention have utility as immunogenic compositions and vaccines.

One aspect of the invention provides an attenuated streptococcal bacteria comprising a functional mutation of a virulence gene selected from the group consisting of *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908 treR, sp0843, endoD, neuB, pgdA, glnQ,* and *nadR*, wherein said functional mutation attenuates the bacteria.

The functionally mutated genes enumerated above may comprise a polynucleotide selected from the group consisting of:

a) a polynucleotide sequence selected from group consisting of SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21,23, 142,144,146,148,150, and 152;

b) a polynucleotide that hybridizes to the complement of a polynucleotide sequence set forth in a) under moderate to highly stringent conditions; and

c.) a polynucleotide that encodes a polypeptide that has at least 99%, 98% 97% 96% 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86% 85%, 84%, 83%, 82%, 81% 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71% 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60% identity and/or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22,24,143,145,147,149,151, and 153.

Also contemplated are immunogenic compositions, preferably vaccine compositions, comprising the attenuated bacteria of the invention, optionally comprising a suitable adjuvant and/or a pharmaceutically acceptable diluent or carrier. In order for a modified strain to be effective in a vaccine formulation, the attenuation must be significant enough to prevent the pathogen from evoking severe clinical symptoms, but also insignificant enough to allow limited replication and growth of the bacteria in the host.

The invention also provides polynucleotides encoding gene products that are required for virulence in gram-positive bacteria, particularly streptococci . Polynucleotides of the invention include DNA, such as complementary DNA,

genomic DNA, including complementary or anti-sense DNA, and wholly or partially synthesized DNA; RNA, including sense and antisense strands; and peptide nucleic acids as described, for example in Corey, *TIBTECH 15*:224-229 (1997).

Virulence gene polynucleotides of the invention include an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide sequence set forth in SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23, 142,144,146,148,150, and 152;

(b) a polynucleotide that encodes a polypeptide that has at least 99%, 98% 97% 96% 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86% 85%, 84%, 83%, 82%, 81% 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71% 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60% identity and or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22 and 24;

(c) a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NOs: SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153;

(d) a polynucleotide which is the complement of any of (a), (b) or (c)

This of course includes species homologs and clonal variants of SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23, 142,144,146,148,150, and 152.

. The invention therefore comprehends gene sequences from other streptococci as well as related gene sequences from other gram-positive bacterial organisms, including naturally occurring (*i.e.* i.e. clonal variants) and artificially induced variants thereof. Knowledge of the sequence of a polynucleotide of the invention makes readily available every possible fragment of that polynucleotide. The invention therefore provides fragments of a polynucleotide of the invention.

The invention further embraces expression constructs comprising polynucleotides of the invention. Host cells transformed, transfected or electroporated with a polynucleotide of the invention are also contemplated. The invention provides methods to produce a polypeptide encoded by a polynucleotide of the invention comprising the steps of growing a host cell of the invention under conditions that permit, and preferably promote, expression of a gene product encoded by the polynucleotide, and isolating the gene product from the host cell or the medium

of its growth.

Identification of polynucleotides of the invention makes available the encoded polypeptides. The invention therefore comprises an isolated polypeptide comprising a polypeptide that has at least 99%, 98% 97% 96% 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86% 85%, 84%, 83%, 82%, 81% 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71% 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60% identity and or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153.

Polypeptides of the invention include full length and fragment, or truncated, proteins; variants thereof; fusion, or chimeric proteins; and analogs, including those wherein conservative amino acid substitutions have been introduced into wild-type polypeptides.

Antibodies that specifically recognize polypeptides of the invention are also provided, and include monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, as well as compounds that include CDR sequences which specifically recognize a polypeptide of the invention. The invention also provides anti-idiotype antibodies immunospecific for antibodies of the invention.

According to another aspect of the invention, methods are provided for identifying novel anti-bacterial agents that modulate the function of gram-positive bacterial virulence genes or gene products. Methods of the invention include screening potential agents for the ability to interfere with expression or activity of one or more gene products selected from the group consisting of *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908, treR sp0843, endoD, neuB, pgdA, glnQ,* and *nadR*,

Also contemplated are methods for screening potential agents for the ability to interfere with biological function of a bacterial gene product encoded one or more streptococcal genes selected from the group consisting of *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908, treR, sp0843, endoD, neuB, pgdA, glnQ,* and *nadR*, followed by identifying agents that provide positive results in such screening assays. In particular, agents that interfere with the expression of

virulence gene products include anti-sense polynucleotides and ribozymes that are complementary to the virulence gene sequences. The invention further embraces methods to modulate transcription of gene products of the invention through use of oligonucleotide-directed triplet helix formation.

Agents that interfere with the function of virulence gene products include variants of virulence gene products, binding partners of the virulence gene products and variants of such binding partners, and enzyme inhibitors (where the product is an enzyme).

Novel anti-bacterial agents identified by the methods described herein are provided, as well as methods for treating a subject suffering from infection with gram positive bacteria involving administration of such novel anti-bacterial agents in an amount effective to reduce bacterial presence.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the invention which describes presently prepared embodiments thereof.

DETAILED DESCRIPTION OF THE INVENTION

Some General Definitions

In the discussion below we will often make use of the term "identity" or similarity as applied to the amino acid sequences of polypeptides. Percent amino acid sequence "identity" with respect to polypeptides is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the target sequences after aligning both sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percent sequence identity is determined by conventional methods. For example, BLASTP 2.2.6 [Tatusova TA and TL Madden, "BLAST 2 sequences- a new tool for comparing protein and nucleotide sequences." (1999) FEMS Microbiol Lett. **174:**247-250.]

Briefly, as noted above, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 0.1, and the "blosum62" scoring matrix of Henikoff and Henikoff (Proc. Nat. Acad. Sci. USA 89:10915-10919. 1992).

The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence + number of gaps introduced into the longer sequence to align the two sequences]}} \times 100$$

Percent sequence "similarity" (often referred to as "homology") with respect to a polypeptide of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the target sequences after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity (as described above), and also considering any conservative substitutions as part of the sequence identity.

$$\frac{\text{Total number of identical matches and conservative substitutions}}{\text{[length of the longer sequence +number of gaps introduced into the longer sequence to align the two sequences]}} \times 100$$

Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties.

Exemplary conservative substitutions are set out in Table 1.

Table 1

Amino Acid	**Conservative Changes**
Alanine (A)	Glycine (G), Serine (S)
Aspartic Acid (D)	Glutamic Acid (E)
Glutamic Acid (E)	Aspartic Acid (D)
Phenylalanine (F)	Tryptophan (W), Tyrosine (Y)
Glycine (G)	Alanine (A)
Histidine (H)	Tyrosine (Y)
Isoleucine (I)	Leucine (L), Methionine (M), Valine (V)
Lysine (K)	Arginine (R)
Leucine (L)	Isoleucine (1), Methionine (M) Valine (V)
Methionine (M)	Isoleucine (1), Leucine (L), Valine (V)
Asparagine (N)	Glutamine (Q)
Glutamine (Q)	Asparagine (N)
Arginine (R)	Lysine (K)
Serine (S)	Alanine (A), Threonine (T)

Threonine (T)	Serine (S)
Valine (V)	Isoleucine (I), Methionine (M) Valine (V)
Tryptophan (W)	Phenylalanine (F), Tyrosine (Y)
Tyrosine (Y)	Phenylalanine (F) Histidine (H) Tryptophan (W)

As used hereinafter "isolated" means altered by the hand of man from the natural state. If an "isolated" composition or substance 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 animal 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.

This application makes reference to sequences which "hybridize under moderate to highly stringent conditions" to the non-coding strand, or complement, of any one of the polynucleotides of the invention. Exemplary high stringency conditions include a final wash in buffer comprising 0.2X SSC/0.1% SDS, at 65°C to 75°C, while exemplary moderate stringency conditions include a final wash in buffer comprising 2X SSC/0.1% SDS, at 35°C to 45°C. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described in Ausubel, *et al.* (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, *et al.*, (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

"Virulence genes," as used herein, are genes whose function or products are required for successful establishment and/or maintenance of a bacterial population within a host animal. Thus, virulence genes and/or the proteins encoded thereby may be involved in, but not essential for, growth of the organism in the host organism; they may also be involved in pathogenesis in the host organism. We have identified *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908* , *treR sp0843, endoD, neuB, pgdA, glnQ,* and *nadR*,as virulence genes in the streptococci.

The activities of the proteins encoded by the virulence genes we have

identified are discussed below.

GuaB encodes an IMP dehydrogenase (EC1.1.1.205), which converts inosine 5'-phosphate to xanthosine 5'-phosphate. GuaA encodes a GMP synthetase (EC6.3.4.1) which converts xanthosine 5'-phosphate to GMP. These two enzymes function in *de novo* synthesis of GMP and in the salvage of purine nucleotides. (Neidhart, F.C., Ed. in Chief. *Escherichia coli* and *Salmonella* Cellular and Molecular Biology, 2nd Edition. 1996. ASM Press, Washington, D.C.; and Noriega FR., Losonsky G., Lauderbaugh C., Liao FM., Wang JY., and Levine MM. 1996. "Engineered deltaguaB-A delta virG *Shigella flexneri* 2a strain CVD 1205: construction, safety, immunogenicity, and potential efficacy as a mucosal vaccine." Infect. Immun. 64(8):3055-61.)

PurA encodes adenylosuccinate synthetase (EC6.3.4.4) which converts IMP to N^6-(1,2-dicarboxyethyl)-AMP. This enzyme is used in *de novo* synthesis of AMP and in the salvage of purine nucleotides. (Neidhart, F.C., Ed. in Chief. *Escherichia coli* and *Salmonella* Cellular and Molecular Biology, 2nd Edition. 1996. ASM Press, Washington, D.C.; and Dunstan SJ. Simmons CP. and Strugnell RA. 1998. "Comparison of the abilities of different attenuated *Salmonella typhimurium* strains to elicit humoral immune responses against a heterologous antigen." Infect. Immun. 66(2):732-40.)

PurD encodes GAR synthetase (EC6.3.4.13) which converts 5-phospho-D-ribosylamine + glycine to N^1-(5-phospho-D-ribosyl)glycinamide. This is an early step in the *de novo* synthesis of IMP, which can then feed into the GuaBA and PurA enzymatic reactions to produce GMP or AMP, respectively. (Neidhart, F.C., Ed. in Chief. *Escherichia coli* and *Salmonella* Cellular and Molecular Biology, 2nd Edition. 1996. ASM Press, Washington, D.C.; and Baumler AJ., Kusters JG., Stojiljkovic I., and Heffron F. 1994. "Salmonella typhimurium loci involved in survival within macrophages." Infect. Immun. 62(5):1623-30.)

ManM encodes the IIC membrane component of a mannose-specific phosphotransferase system that transports across the bacterial membrane and phosphorylates mannose. The phosphotransferase systems in general are implicated in the transport of several carbohydrates for use as carbon sources. (Neidhart, F.C., Ed. In Chief. Escherichia coli and Salmonella Cellular and Molecular Biology, 2nd Edition. 1996. ASM Press, Washington, D.C.; and Cochu A., Vadeboncoeur C.,

Moineau S., and Frenette M. 2003. "Genetic and biochemical characterization of the phosphoenolpyruvate:glucose/mannose phosphotransferase system of Streptococcus thermophilus." App.Environ. Micro. 69(9):5423-32; and Lortie LA., Pelletier M., Vadeboncoeur C., and Frenette M. 2000 "The gene encoding IIAB(Man)L in Streptococcus salivarius is part of a tetracistronic operon encoding a phosphoenolpyruvate: mannose/glucose phosphotransferase system." Microbiology. 146 (Pt 3):677-85.)

ScrB encodes a sucrose-6-phosphate hydrolase (EC 3.2.1.26) which catalyzes the hydrolysis of terminal non-reducing β-D-fructofuranoside residues in β-D-fructofuranosides or the breakdown of sucrose-6-phosphate into glucose-6-phosphate and fructose. This is a part of the catabolic pathway for the utilization of sucrose. (Neidhart, F.C., Ed. In Chief. Escherichia coli and Salmonella Cellular and Molecular Biology, 2nd Edition. 1996. ASM Press, Washington, D. C.; and Bogs J. and Geider K. 2000. "Molecular analysis of sucrose metabolism of *Erwinia amylovora* and influence on bacterial virulence." J. Bact.. 182(19):5351-8; and Garsin DA., Sifri CD., Mylonakis E., Qin X., Singh KV., Murray BE., Calderwood SB., and Ausubel FM. 2001. "A simple model host for identifying Gram-positive virulence factors." PNAS. 98(19):10892-7).

ScrR is a member of the GalR-LacI repressor family and is a regulator of the sucrose regulon, which includes multiple *scr* genes. ScrR is a repressor that is responsive to intracellular fructose and fructose 1-phosphate. (Neidhart, F.C., Ed. in Chief. *Escherichia coli* and *Salmonella* Cellular and Molecular Biology, 2nd Edition. 1996. ASM Press, Washington, D.C.; and Bogs J. Geider K. 2000. "Molecular analysis of sucrose metabolism of *Erwinia amylovora* and influence on bacterial virulence." J. Bact.. 182(19):5351-8; and Garsin DA., Sifri CD., Mylonakis E., Qin X., Singh KV., Murray BE., Calderwood SB. and Ausubel FM. 2001. "A simple model host for identifying Gram-positive virulence factors." PNAS. 98(19):10892-7; and Hiratsuka,K., Wang,B., Sato,Y. and Kuramitsu, H. 1998. "Regulation of sucrose-6-phosphate hydrolase activity in Streptococcus mutans: characterization of the *scrR* gene." Infect. Immun. 66 (8), 3736-3743.)

GtfA (EC 2.4.1.7) is a sucrose phosphorylase that catalyzes the reaction of sucrose + phosphate to D-fructose + α-D-glucose 1-phosphate. (Narimatsu M. Noiri

Y. Itoh S. Noguchi N. Kawahara T. Ebisu S. 2004. "Essential role for the *gtfA* gene encoding a putative glycosyltransferase in the adherence of *Porphyromonas gingivalis*." Infect. Immun. 72(5):2698-702; and Barletta RG., Michalek SM., and Curtiss R III. 1988. "Analysis of the virulence of Streptococcus mutans serotype c gtfA mutants in the rat model system." Infect. Immun. 56(2):322-30; and Russell RR, Mukasa H, Shimamura A, Ferretti JJ. 1988. "*Streptococcus mutans gtfA* gene specifies sucrose phosphorylase." Infect Immun. 56(10):2763-5.)

TreR is a transcriptional repressor of the trehalose operon.

SpyM3-0908 is a putative amino acid ABC transporter that has some similarity to glutamine transporters.

Spr1018 is a conserved hypothetical protein with unknown function.

Smu.61 is a conserved hypothetical, putative transcriptional regulator.

Lin0523 is a *Listeria innocua* sequence of unknown function.

Sp0843 is a putative deoxyribose-phosphate aldolase.

EndoD is a endo-beta-N-aceytlglucosaminidase.

NeuB is a putative N-acetyl neuramic acid synthetase.

PgdA is a peptidoglycan GlcNAc deacetylase.

GlnQ is an ABC transporter involved in glutamine transport, encoding the ATP-binding protein.

NadR is a transcriptional regulator.

By way of example, the genes enumerated above may comprise a polynucleotide selected from the group consisting of:

a) a polynucleotide sequence selected from group consisting of SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23, 142,144,146,148,150, and 152;

b) a polynucleotide that hybridizes to the complement of a polynucleotide sequence set forth in a) under moderate to highly stringent conditions; or

c.) a polynucleotide that encodes a polypeptide that has at least 99%, 98% 97% 96% 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86% 85%, 84%, 83%, 82%, 81% 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71% 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60% identity and/or similarity to a polypeptide selected

from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153.

"Streptococci", "streptococcal bacteria", and species in the genus *Streptococcus* include, but are not limited to, *S. acidominimus*, *S. agalactiae, S. bovis, S. equinus, S. canis, S. cricetus, S. downei, S. dysgalactiae, S. entericus, S. equi, S. equinus, S. equisimilis, S. gallinaceus, S. iniae, S. mutans, S. parauberis, S. pluranimalium, S. porcinus, S. pyogenes, S. pneumoniae, S. sobrinus, S. uberis, S. suis,* and *S. zooepidemicus*.

"Lancefield serologic grouping", a step in streptococcal identification, is determined by cell wall polysaccharides (C substance, group antigen) Groups are designated by capital letters. Lancefield group A Streptococcus (GAS, Streptococcus pyogenes), are infections are common among children, causing nasopharyngeal infections and complications, thereof. Cattle are often susceptible to GAS, and they are a frequent causative organism of mastitis. Lancefield group B Streptococcus (GBS) are most often seen in cattle, causing mastitis. However, human infants are susceptible as well, often with fatal consequences. Group B streptococci (GBS) constitute a major cause of bacterial sepsis and meningitis among human neonates born in the United States and Western Europe, and are emerging as significant neonatal pathogens in developing countries as well. Lancefield group C infections, such as those caused by *S. equi, S. zooepidemicus*, *S. dysgalactiae*, and others are mainly seen in horses, cattle and pigs, but can also cross the species barrier to humans. Lancefield group D infections are found in all mammals and some birds, sometimes resulting in endocarditis or septicaemia. Lancefield groups E, G, L, P, U and V (*S. porcinus, S, canis, S. dysgalactiae*) are found in various hosts, causing neonatal infections, nasopharyngeal infections, or mastitis. Within Lancefield groups R, S, and T, (and with ungrouped types) *S. suis* is found, an important cause of meningitis, septicemia, arthritis, and sudden death in young pigs. *S. suis* can also cause meningitis in man.There also exists a number of ungrouped streptococcal species, such as *S. mutans*, causing caries in humans, *S. uberis*, causing mastitis in cattle, and *S. pneumonia*, causing major invasive diseases such as pneumonia, bacteremia, and meningitis in humans.

"Signature-tagged mutagenesis (STM)," as used herein, is a method generally described in International Patent Publication No. WO 96/17951, incorporated herein

by reference, and includes, for example, a method for identifying bacterial genes required for virulence in a murine model of bacteremia. In this method, bacterial strains that each have a random mutation in the genome are produced using transposon integration. Each insertional mutation carries a different DNA signature tag which allows mutants to be differentiated from each other. The tags comprise 40 bp variable central regions flanked by invariant "arms" of 20 bp which allow the central portions to be co-amplified by polymerase chain reaction (PCR). Tagged mutant strains are assembled in microtiter dishes, then combined to form the "inoculum pool" for infection studies. At an appropriate time after inoculation, bacteria are isolated from the animal and pooled to form the "recovered pool." The tags in the recovered pool and the tags in the inoculum pool are separately amplified, labeled, and then used to probe filters arrayed with all of the different tags representing the mutants in the inoculum. Mutant strains with attenuated virulence are those which cannot be recovered from, or whose recovery is reduced in, the infected animal, *i.e.,* strains with tags that give strong hybridization signals when probed with tags from the inoculum pool but not when probed with tags from the recovered pool. In a variation of this method, longer tags and non-radioactive detection methods such as chemiluminescence can be used.

Signature-tagged mutagenesis allows a large number of insertional mutant strains to be screened simultaneously in a single animal for loss of virulence. Screening 30 pools of mutant *S. suis* strains resulted in the identification of 58 strains with reduced virulence. Of these, mutations in 13 genes results in significantly attenuated *S. suis* strains. The nucleotide sequence of the open reading frame disrupted by the transposon insertion was determined by sequencing both DNA strands, and an encoded amino acid sequence was deduced. The putative identity of both the polynucleotide and amino acid sequences was determined by comparison of the sequences with DNA and protein database sequences. The sequence information also permitted identification of species homologs or clonal variants in other streptococci by database mining.

A "functional mutation" of a particular virulence gene may occur in protein coding regions of a virulence gene of the invention, as well as in regulatory regions or genes that modulate transcription of the virulence gene mRNA. Functional mutations that decrease expression and/or biological activity of a gene product include

insertions, deletions, or point mutations in the protein coding region of the gene itself or in sequences responsible for, or involved in, control of target gene expression, including non-coding regulatory sequences or regulatory genes themselves. Deletion mutants include those wherein all or part of a specific sequence, coding or non-coding, is deleted.

Attenuated Strains and Vaccines

The identification of gram-positive bacterial, and more particularly *S. suis*, virulence genes provides for microorganisms exhibiting reduced virulence (*i.e.*, attenuated strains), which are useful in vaccines and immunogenic compositions.

One aspect of the invention provides an attenuated streptococcal bacteria comprising a functional mutation of a virulence gene selected from the group consisting of *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908, treR, sp0843, endoD, neuB, pgdA, glnQ,* and *nadR*, wherein said functional mutation attenuates the bacteria.

The functionally mutated genes enumerated above may comprise a polynucleotide selected from the group consisting of:

a) a polynucleotide sequence selected from group consisting of SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23, 142,144,146,148,150, and 152;

b) a polynucleotide that hybridizes to the complement of a polynucleotide sequence set forth in a) under moderate to highly stringent conditions; or

c.) a polynucleotide that encodes a polypeptide that has at least 99%, 98% 97% 96% 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86% 85%, 84%, 83%, 82%, 81% 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71% 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60% identity and/or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153.

The worker of ordinary skill in the art will realize that a "functional mutation" may occur in protein coding regions of a virulence gene of the invention, as well as in regulatory regions or genes that modulate transcription of the virulence gene mRNA. Functional mutations result in decreased expression and/or biological activity of a gene product include insertions, deletions, or point mutations. Such functional mutations are easily screened because they result in a reduction in virulence as described herein.

The worker of ordinary skill will also appreciate that attenuated streptococcal strains of the invention include those bearing more than one functional mutation. More than one mutation may result in additive or synergistic degrees of attenuation. Multiple mutations can be prepared by design or may fortuitously arise from a deletion event originally intended to introduce a single mutation. An example of an attenuated strain with multiple deletions is a *S. suis* strain wherein the *gtfA* and *guaB* genes are functionally deleted.

Identification of virulence genes in comprising SEQ ID NOS: 1,3,5,7,9,11,13,15,17,19,21, 23, 142,144,146,148,150, and 152 in *S. suis* provides information regarding similar genes in other streptococcal species. As an example, identification of any of the genes could potentially lead to identification of conserved genes in a diverse number of streptococcal pathogens, including *S. acidominimus, S. agalactiae, S. bovis, S. equinus, S. canis, S. cricetus, S. downei, S. dysgalactiae, S. entericus, S. equi, S. equinus, S. equisimilis, S. gallinaceus, S. iniae, S. mutans, S. parauberis, S. pluranimalium, S. porcinus, S. pyogenes, S. pneumoniae, S. sobrinus, S. uberis, S. suis,* and *S. zooepidemicus.* Using the virulence genes sequences identified in *S. suis,* similar or homologous genes can be identified in other organisms, particularly within the streptococci, as well as other gram-positive species. Southern hybridization, using the streptococcal genes as probes, can identify related genes in chromosomal libraries derived from other organisms. Alternatively, PCR can be equally effective in gene identification across species boundaries. As still another alternative, complementation of, for example, a *S. suis* mutant with a chromosomal library from other species can also be used to identify genes having the same or related virulence activity. Identification of related virulence genes can therefore lead to production of an attenuated strain of the other organism which can be useful as still another vaccine formulation. Examples of *S. suis* genes that have been demonstrated to exist in other species (*e.g. S. agalactiae, S. pyogenes,* and *S. pneumoniae*) include genes *gtfA, guaB, manM, purA, purD, scrB, scrR and spyM3-0908.* It is appreciated also, that even within *S. suis* that strain variations may exist in the sequences of the virulence genes identified. These variants are easily identified by the ordinarily skilled artisan.

Attenuated *S. suis* strains identified using STM are insertional mutants wherein a virulence gene has been rendered non-functional through insertion of

transposon sequences in either the open reading frame or regulatory DNA sequences. These insertional mutants still contain all of the genetic information required for bacterial virulence, and can possibly revert to a pathogenic state by deletion of the inserted transposon. Therefore, in preparing a vaccine formulation, it is desirable to take the information gleaned from the attenuated strain and create a deletion mutant strain wherein some, most, or all of the virulence gene sequence is removed, thereby precluding the possibility that the bacteria will revert to a virulent state.

The vaccine properties of an attenuated insertional mutant identified using STM are expected to be the same or similar to those of a bacteria bearing a deletion in the same gene. However, it is possible that an insertion mutation may exert "polar" effects on adjoining gene sequences, and as a result, the insertion mutant may possess characteristic distinct from a mutant strain with a deletion in the same gene sequence. Deletion mutants can be constructed using any of a number of techniques well known and routinely practiced in the art.

In one example, a strategy using counterselectable markers can be employed which has commonly been utilized to delete genes in many bacteria. For a review, see, for example, Reyrat, *et al., Infection and Immunity 66*:4011-4017 (1998), incorporated herein by reference. In this technique, a double selection strategy is often employed, wherein a plasmid is constructed encoding both a selectable and counterselectable marker, with flanking DNA sequences derived from both sides of the desired deletion. The selectable marker is used to select for bacteria in which the plasmid has integrated into the genome in the appropriate location and manner. The counterselecteable marker is used to select for the very small percentage of bacteria that have spontaneously eliminated the integrated plasmid. A fraction of these bacteria will then contain only the desired deletion with no other foreign DNA present. The key to the use of this technique is the availability of a suitable counterselectable marker.

In another technique, the *cre-lox* system is used for site-specific recombination of DNA. The system consists of 34 base pair *lox* sequences that are recognized by the bacterial *cre* recombinase gene. If the *lox* sites are present in the DNA in an appropriate orientation, DNA flanked by the *lox* sites will be excised by the *cre* recombinase, resulting in the deletion of all sequences except for one remaining copy of the *lox* sequence. Using standard recombination techniques, it is possible to delete

the targeted gene of interest in the streptococcal genome and to replace it with a selectable marker (*e.g.*, a gene coding for kanamycin resistance) that is flanked by the *lox* sites. Transient expression (by electroporation) of a suicide plasmid containing the *cre* gene under control of a promoter that functions in streptococcal species of interest of the *cre* recombinase should result in efficient elimination of the *lox*-flanked marker. This process would result in a mutant containing the desired deletion and one copy of the *lox* sequence.

In another approach, it is possible to directly replace a desired deleted sequence in the genome of the streptococcal species of interest with a marker gene, such as green fluorescent protein (GFP), β-galactosidase, or luciferase. In this technique, DNA segments flanking a desired deletion are prepared by PCR and cloned into a suicide (non-replicating) vector for streptococcal species of interest. An expression cassette, containing a promoter active in streptococci and the appropriate marker gene, is cloned between the flanking sequences. The plasmid is introduced into wild-type streptococcal strain. Bacteria that incorporate and express the marker gene (probably at a very low frequency) are isolated and examined for the appropriate recombination event (*i.e.* replacement of the wild type gene with the marker gene).

The present invention provides combination vaccines suitable for administration to a subject animal. The combination vaccines of the present invention include an attenuated streptococcal strain in combination with at least one other antigen capable of inducing a protective immune response in the subject animal against disease caused by such other antigen. Preferred combination vaccines of the present invention include, but are not limited to, porcine reproductive and respiratory syndrome virus, pseudorabies virus, porcine circovirus type II, swine influenza virus, *Salmonella cholerasuis*, *Salmonella typhimurium*, *Erysipelothrix rhusiopathiae*, *Lawsonia intracellularis*, *Haemophilus parasuis*, *Bordetella bronchiseptica*, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Escherichia coli*, *Pasteurella multocida*, and *Clostridium perfringens* type A and type C.

The reduced virulence of these organisms and their immunogenicity may be confirmed by administration to a subject animal. While it is possible for an avirulent microorganism of the invention to be administered alone, one or more of such mutant microorganisms are preferably administered in a vaccine composition containing suitable adjuvant(s) and pharmaceutically acceptable diluent(s) or carrier(s). The

carrier(s) must be "acceptable" in the sense of being compatible with the avirulent microorganism of the invention and not deleterious to the subject to be immunized. Typically, the carriers will be water or saline which will be sterile and pyrogen-free. The subject to be immunized is one needing protection from a disease caused by a virulent form of streptococci or other pathogenic microorganisms.

Any adjuvant known in the art may be used in the vaccine composition, including oil-based adjuvants such as Freund's Complete Adjuvant and Freund's Incomplete Adjuvant, mycolate-based adjuvants (*e.g.,* trehalose dimycolate), bacterial lipopolysaccharide (LPS), peptidoglycans (*i.e.,* mureins, mucopeptides, or glycoproteins such as N-Opaca, muramyl dipeptide [MDP], or MDP analogs), proteoglycans (*e.g.,* extracted from *Klebsiella pneumoniae*), streptococcal preparations (*e.g.,* OK432), Biostim™ (*e.g.,* 01K2), the "Iscoms" of EP 109 942, EP 180 564 and EP 231 039, aluminum hydroxide, saponin, DEAE-dextran, neutral oils (such as miglyol), vegetable oils (such as arachis oil), liposomes, Pluronic® polyols, the Ribi adjuvant system (see, for example GB-A-2 189 141), or interleukins, particularly those that stimulate cell mediated immunity. An alternative adjuvant consisting of extracts of *Amycolata*, a bacterial genus in the order Actinomycetales, has been described in U.S. Patent No. 4,877,612. Other adjuvants include mineral gels, e.g., aluminum hydroxide; surface active substances such as lysolecithin; glycosides, e.g., saponin derivatives such as Quil A or GPI-0100 (United States Patent No. 5,977,081); cationic surfactants such as DDA, pluronic polyols; polyanions; non-ionic block polymers, e.g., Pluronic F-127 (B.A.S.F., USA); peptides; mineral oils, e.g. Montanide ISA-50 (Seppic, Paris, France), carbopol, Amphigen (Hydronics, Omaha, NE USA), Alhydrogel (Superfos Biosector, Frederikssund, Denmark) oil emulsions, e.g. an emulsion of mineral oil such as BayolF/Arlacel A and water, or an emulsion of vegetable oil, water and an emulsifier such as lecithin; alum, cholesterol, rmLT, cytokines and combinations thereof. Additionally, proprietary adjuvant mixtures are commercially available. The immunogenic component may also be incorporated into liposomes, or conjugated to polysaccharides and/or other polymers for use in a vaccine formulation.

The adjuvant used will depend, in part, on the recipient organism. The amount of adjuvant to administer will depend on the type and size of animal. Optimal dosages may be readily determined by routine methods.

The vaccine compositions optionally may include vaccine-compatible pharmaceutically acceptable (*i.e.,* sterile and non-toxic) liquid, semisolid, or solid diluents that serve as pharmaceutical vehicles, excipients, or media. Any diluent known in the art may be used. Exemplary diluents include, but are not limited to, polyoxyethylene sorbitan monolaurate, magnesium stearate, methyl- and propylhydroxybenzoate, talc, alginates, starches, lactose, sucrose, dextrose, sorbitol, mannitol, gum acacia, calcium phosphate, mineral oil, cocoa butter, and oil of theobroma.

The vaccine compositions can be packaged in forms convenient for delivery. The compositions can be enclosed within a capsule, caplet, sachet, cachet, gelatin, paper, or other container. These delivery forms are preferred when compatible with entry of the immunogenic composition into the recipient organism and, particularly, when the immunogenic composition is being delivered in unit dose form. The dosage units can be packaged, *e.g.,* in tablets, capsules, suppositories or cachets.

The vaccine compositions may be introduced into the subject to be immunized by any conventional method including, *e.g.,* by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, or subcutaneous injection; by oral, sublingual, nasal, anal, or vaginal, delivery. The treatment may consist of a single dose or a plurality of doses over a period of time.

The invention also comprehends use of an attenuated bacterial strain of the invention for manufacture of a vaccine medicament to prevent or alleviate bacterial infection and/or symptoms associated therewith. The invention also provides use of inhibitors of the invention for manufacture of a medicament to prevent or alleviate bacterial infection and/or symptoms associated therewith.

It will be appreciated that the vaccine of the invention may be useful in the fields of human medicine and veterinary medicine. Thus, the subject to be immunized may be a human or other animal, for example, farm animals including cows, sheep, pigs, horses, goats and poultry (*e.g.*, chickens, turkeys, ducks and geese) companion animals such as dogs and cats, exotic and/or zoo animals, and laboratory animals including mice, rats, rabbits, guinea pigs, and hamsters.

Polynucleotides

The invention also provides polypeptides and corresponding polynucleotides required for streptococcal virulence. The invention includes both naturally occurring

and non-naturally occurring polynucleotides and polypeptide products thereof. Naturally occurring virulence products include distinct gene and polypeptide species, as well as corresponding species homologs expressed in organisms other than *S. suis*. Non-naturally occurring virulence products include variants of the naturally occurring products, such as analogs and virulence products which include covalent modifications.

The invention includes isolated genes gene fragments and gene products from the loci *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908, treR, sp0843, endoD, neuB, pgdA, glnQ,* and *nadR,* from *S. suis.*

Preferred DNA sequences are set out in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152, and species homologs or clonal variants thereof. The worker of skill in the art will readily appreciate that the preferred DNA of the invention comprises a double-stranded molecule, for example, molecules having the sequences set forth in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152 and species homologs or clonal variants thereof, along with the complementary molecule (the "non-coding strand" or "complement") having a sequence deducible from the sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152 according to Watson-Crick base pairing rules for DNA. Also preferred are polynucleotides encoding the gene products encoded by any one of the polynucleotides set out in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152 and species homologs or clonal variants thereof.

The invention includes therefore, an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of :

(a) a polynucleotide sequence set forth in SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23, 142,144,146,148,150, and 152; and

(b) a polynucleotide that encodes a polypeptide that has at least 99%, 98% 97% 96% 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86% 85%, 84%, 83%, 82%, 81% 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71% 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60% identity and or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153; and

(c) a polynucleotide encoding a polypeptide selected from the group consisting of

SEQ ID NOs: SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153;

(d) a polynucleotide that hybridizes to the complement of a polynucleotide sequence under moderately or highly stringent conditions.

(e) a polynucleotide which is the complement of any of (a), (b), (c) or (d).

The present invention provides novel isolated streptococcal polynucleotides (e.g., DNA sequences and RNA transcripts, both sense and complementary antisense strands) encoding the bacterial virulence gene products. DNA sequences of the invention include genomic as well as wholly or partially chemically or enzymatically synthesized DNA sequences. Genomic DNA of the invention comprises the protein coding region for a polypeptide of the invention and includes variants that may be found in other bacterial strains of the same species. "Synthesized," as used herein and is understood in the art, refers to purely chemical, as opposed to enzymatic, methods for producing polynucleotides. "Wholly" synthesized DNA sequences are therefore produced entirely by chemical means, and "partially" synthesized DNAs embrace those wherein only portions of the resulting DNA were produced by chemical means.

The polynucleotide sequence information provided by the invention makes possible the identification and isolation of polynucleotides encoding related bacterial virulence molecules and genes which are species homologues by well known techniques including Southern and/or Northern hybridization, polymerase chain reaction (PCR), and database mining. Examples of polypeptides encoded by polynucleotides related to *gtfA, guaB, manM, purA, purD, scrB, scrR,* and *spyM3-0908* are set out in Table 5.

Vectors and Host Cells

Autonomously replicating recombinant expression constructions such as plasmid and viral DNA vectors incorporating virulence gene sequences are also provided. Expression constructs wherein virulence polypeptide-encoding polynucleotides are operatively linked to an endogenous or exogenous expression control DNA sequence and a transcription terminator are also provided. The virulence genes may be cloned by PCR, using streptococcal genomic DNA as the template. For ease of inserting the gene into expression vectors, PCR primers are chosen so that the PCR-amplified gene has a restriction enzyme site at the 5′ end preceding the initiation codon, and a restriction enzyme site at the 3′ end after the

termination codon. If desirable, the codons in the gene are changed, without changing the amino acids, according to the codon preference of the organism being used for expression. Optimization of codon usage may lead to an increase in the expression of the gene product in the expression system. If the gene product is to be produced extracellularly, either in the periplasm of *E. coli* or other bacteria, or into the cell culture medium, the gene is cloned without its initiation codon and placed into an expression vector behind a signal sequence.

According to another aspect of the invention, host cells are provided, including procaryotic and eukaryotic cells, either stably or transiently transformed, transfected, or electroporated with polynucleotide sequences of the invention in a manner which permits expression of virulence polypeptides of the invention. Expression systems of the invention include bacterial, yeast, fungal, viral, invertebrate, and mammalian cells systems. Host cells of the invention are a valuable source of immunogen for development of antibodies specifically immunoreactive with the virulence gene product. Host cells of the invention are conspicuously useful in methods for large scale production of virulence polypeptides wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells or from the medium in which the cells are grown by, for example, immunoaffinity purification or any of the multitude of purification techniques well known and routinely practiced in the art. Any suitable host cell may be used for expression of the gene product, such as *E. coli*, other bacteria, including *Bacillus* and *S. aureus*, yeast, including *Pichia pastoris* and *Saccharomyces cerevisiae*, insect cells, or mammalian cells, including CHO cells, utilizing suitable vectors known in the art. Proteins may be produced directly or fused to a peptide or polypeptide, and either intracellularly or extracellularly by secretion into the periplasmic space of a bacterial cell or into the cell culture medium. Secretion of a protein requires a signal peptide (also known as pre-sequence). A number of signal sequences from prokaryotes and eukaryotes are known to function for the secretion of recombinant proteins. During the protein secretion process, the signal peptide is removed by signal peptidase to yield the mature protein.

Polypeptides

The invention also provides isolated streptococcal virulence polypeptides encoded by a polynucleotide of the invention. Presently preferred are polypeptides

comprising the amino acid sequences encoded by any one of the polynucleotides set out in SEQ ID NOs : 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152 or the polypeptides set forth in SEQ ID NOs: 2,4,6,8,10,12, 14,16,18,20,22, 24,143,145,147,149,151, and 153, and species homologs or clonal variants thereof. The invention embraces virulence polypeptides encoded by a DNA selected from the group consisting of : a) the DNA sequence set out in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152, and species homologs or clonal variants thereof, and b) a DNA molecule, encoding a virulence gene product, that hybridizes under moderately stringent conditions to the DNA of (a). The invention also embraces polypeptides that have at least about 99%, 98% 97% 96% 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86% 85%, 84%, 83%, 82%, 81% 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71% 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, identity and/or homology to the preferred polypeptides of the invention.

Variant virulence products of the invention include mature virulence gene products, *i.e.*, wherein leader or signal sequences are removed, or having additional amino terminal residues. Virulence gene products having an additional methionine residue at position -1 are contemplated, as are virulence products having additional methionine and lysine residues at positions -2 and -1. Variants of these types are particularly useful for recombinant protein production in bacterial cell types. Variants of the invention also include gene products wherein amino terminal sequences derived from other proteins have been introduced, as well as variants comprising amino terminal sequences that are not found in naturally occurring proteins.

The invention also embraces variant polypeptides having additional amino acid residues which result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as a fusion protein with glutathione-S-transferase (GST) provide the desired polypeptide having an additional glycine residue at position -1 following cleavage of the GST component from the desired polypeptide. Variants which result from expression using other vector systems are also contemplated.

To simplify the protein purification or isolation process, a purification tag may be added either at the 5′ or 3′ end of the gene coding sequence. Commonly used purification tags include a stretch of six histidine residues (U.S. Patent Nos. 5,284,933

and 5,310,663), a streptavidin-affinity tag described by Schmidt and Skerra, *Protein Engineering, 6*:109-122 (1993), a FLAG peptide [Hopp *et al.*, *Biotechnology, 6*:1205-1210 (1988)], glutathione S-transferase [Smith and Johnson, *Gene, 67*:31-40 (1988)], and thioredoxin [LaVallie *et al.*, *Bio/Technology, 11*:187-193 (1993)]. To remove these peptides or polypeptides, a proteolytic cleavage recognition site may be inserted at the fusion junction. Commonly used proteases are factor Xa, thrombin, and enterokinase.

Antibodies

Also contemplated by the present invention are antibodies (*e.g.*, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, humanized, human, and CDR-grafted antibodies, including compounds which include CDR sequences which specifically recognize a polypeptide of the invention) and other binding proteins specific for virulence gene products or fragments thereof. The term "specific for" indicates that the variable regions of the antibodies of the invention recognize and bind a virulence polypeptide exclusively (*i.e.*, are able to distinguish a single virulence polypeptides from related virulence polypeptides despite sequence identity, homology, or similarity found in the family of polypeptides), but may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see Harlow *et al.* (Eds), Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory; Cold Spring Harbor , NY (1988), Chapter 6. Antibodies that recognize and bind fragments of the virulence polypeptides of the invention are also contemplated, provided that the antibodies are first and foremost specific for, as defined above, a virulence polypeptide of the invention from which the fragment was derived.

Modulation or Manipulation of Expression of the Nuceic Acids and Polypeptides of the Invention

The DNA and amino acid sequence information provided by the present invention also makes possible the systematic analysis of the structure and function of the virulence genes and their encoded gene products. Knowledge of a polynucleotide

encoding a virulence gene product of the invention also makes available anti-sense polynucleotides which recognize and hybridize to polynucleotides encoding a virulence polypeptide of the invention. Full length and fragment anti-sense polynucleotides are provided. The worker of ordinary skill will appreciate that fragment anti-sense molecules of the invention include (i) those which specifically recognize and hybridize to a specific RNA (as determined by sequence comparison of DNA encoding a virulence polypeptide of the invention to DNA encoding other known molecules), as well as (ii) those which recognize and hybridize to RNA encoding variants of the family of virulence proteins. Antisense polynucleotides that hybridize to RNA encoding other members of the virulence family of proteins are also identifiable through sequence comparison to identify characteristic, or signature, sequences for the family of molecules.

The invention further contemplates methods to modulate gene expression through use of ribozymes. For a review, see Gibson and Shillitoe, *Mol. Biotech.* 7:125-137 (1997). Ribozyme technology can be utilized to inhibit translation of mRNA in a sequence specific manner through (i) the hybridization of a complementary RNA to a target mRNA and (ii) cleavage of the hybridized mRNA through nuclease activity inherent to the complementary strand. Ribozymes can be identified by empirical methods but more preferably are specifically designed based on accessible sites on the target mRNA [Bramlage, *et al., Trends in Biotech 16*:434-438 (1998)]. Delivery of ribozymes to target cells can be accomplished using either exogenous or endogenous delivery techniques well known and routinely practiced in the art. Exogenous delivery methods can include use of targeting liposomes or direct local injection. Endogenous methods include use of viral vectors and non-viral plasmids.

Ribozymes can specifically modulate expression of virulence genes when designed to be complementary to regions unique to a polynucleotide encoding a virulence gene product. "Specifically modulate" therefore is intended to mean that ribozymes of the invention recognizes only a single polynucleotide. Similarly, ribozymes can be designed to modulate expression of all or some of a family of proteins. Ribozymes of this type are designed to recognize polynucleotide sequences conserved in all or some of the polynucleotides which encode the family of proteins.

The invention further embraces methods to modulate transcription of a

virulence gene of the invention through use of oligonucleotide-directed triplet helix formation. For a review, see Lavrovsky, *et al., Biochem. Mol. Med. 62*:11-22 (1997). Triplet helix formation is accomplished using sequence specific oligonucleotides which hybridize to double stranded DNA in the major groove as defined in the Watson-Crick model. Hybridization of a sequence specific oligonucleotide can thereafter modulate activity of DNA-binding proteins, including, for example, transcription factors and polymerases. Preferred target sequences for hybridization include transcriptional regulatory regions that modulate virulence gene product expression. Oligonucleotides which are capable of triplet helix formation are also useful for site-specific covalent modification of target DNA sequences. Oligonucleotides useful for covalent modification are coupled to various DNA damaging agents as described in Lavrovsky, *et al.* [*supra*].

Methods of Identifying Anti-Bacterial Agents.

The identification of streptococcal virulence genes renders the genes and gene products useful in methods for identifying anti-bacterial agents. Such methods include assaying potential agents for the ability to interfere with expression of virulence gene products represented by the DNA sequences set forth in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152, and species homologs or clonal variants thereof (*i.e.*, the genes represented by DNA sequences of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152 encode the virulence gene product, or the DNA sequences of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152 are adjacent the gene encoding the virulence gene product, or are involved in regulation of expression of the virulence gene product), or assaying potential agents for the ability to interfere with the function of a bacterial gene product encoded in whole or in part by a DNA sequence set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 142,144,146,148,150, and 152, species homologs or clonal variants thereof, or the complementary strand thereof, followed by identifying agents that are positive in such assays. Polynucleotides and polypeptides useful in these assays include not only the genes and encoded polypeptides as disclosed herein, but also variants thereof that have substantially the same activity as the wild-type genes and polypeptides.

The virulence gene products produced by the methods described above are

used in high throughput assays to screen for inhibitory agents. The sources for potential agents to be screened are chemical compound libraries, fermentation media of *Streptomycetes,* other bacteria and fungi, and cell extracts of plants and other vegetations. For proteins with known enzymatic activity, assays are established based on the activity, and a large number of potential agents are screened for ability to inhibit the activity. For proteins that interact with another protein or nucleic acid, binding assays are established to measure such interaction directly, and the potential agents are screened for ability to inhibit the binding interaction.

The use of different assays known in the art is contemplated according to this aspect of the invention. When the function of the virulence gene product is known or predicted by sequence similarity to a known gene product, potential inhibitors can be screened in enzymatic or other types of biological and/or biochemical assays keyed to the function and/or properties of the gene product. When the virulence gene product is known or predicted by sequence similarity to a known gene product to interact with another protein or nucleic acid, inhibitors of the interaction can be screened directly in binding assays. The invention contemplates a multitude of assays to screen and identify inhibitors of binding by the virulence gene product. In one example, the virulence gene product is immobilized and interaction with a binding partner is assessed in the presence and absence of a putative inhibitor compound. In another example, interaction between the virulence gene product and its binding partner is assessed in a solution assay, both in the presence and absence of a putative inhibitor compound. In both assays, an inhibitor is identified as a compound that decreases binding between the virulence gene product and its binding partner. Other assays are also contemplated in those instances wherein the virulence gene product binding partner is a protein. For example, variations of the di-hybrid assay are contemplated wherein an inhibitor of protein/protein interactions is identified by detection of a positive signal in a transformed or transfected host cell as described in PCT publication number WO 95/20652, published August 3, 1995.

Candidate inhibitors contemplated by the invention include compounds selected from libraries of potential inhibitors. There are a number of different libraries used for the identification of small molecule modulators, including: (1) chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules. Chemical

libraries consist of structural analogs of known compounds or compounds that are identified as "hits" or "leads" via natural product screening. Natural product libraries are collections of microorganisms, animals, plants, or marine organisms which are used to create mixtures for screening by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of plants or marine organisms. Natural product libraries include polyketides, non-ribosomal peptides, and variants (non-naturally occurring) thereof. For a review, see *Science 282*:63-68 (1998). Combinatorial libraries are composed of large numbers of peptides, oligonucleotides, or organic compounds as a mixture. They are relatively easy to prepare by traditional automated synthesis methods, PCR, cloning, or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, *Curr. Opin. Biotechnol. 8*:701-707 (1997). Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to modulate activity.

Still other candidate inhibitors contemplated by the invention can be designed and include soluble forms of binding partners, as well as binding partners as chimeric, or fusion, proteins. Binding partners as used herein broadly encompasses antibodies, antibody fragments, and modified compounds comprising antibody domains that are immunospecific for the expression product of the identified virulence gene.

Other assays may be used when a binding partner (*i.e.,* ligand) for the virulence gene product is not known, including assays that identify binding partners of the target protein through measuring direct binding of test binding partner to the target protein, and assays that identify binding partners of target proteins through affinity ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods. Alternatively, such binding interactions are evaluated indirectly using the yeast two-hybrid system described in Fields and Song, *Nature, 340*:245-246 (1989), and Fields and Sternglanz, *Trends in Genetics, 10*:286-292 (1994), both of which are incorporated herein by reference. The two-hybrid system is a genetic assay for detecting interactions between two proteins or polypeptides. It can be used to identify proteins that bind to a known protein of

interest, or to delineate domains or residues critical for an interaction. Variations on this methodology have been developed to clone genes that encode DNA-binding proteins, to identify peptides that bind to a protein, and to screen for drugs. The two-hybrid system exploits the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA-binding domain that binds to an upstream activation sequence (UAS) of a reporter gene, and is generally performed in yeast. The assay requires the construction of two hybrid genes encoding (1) a DNA-binding domain that is fused to a first protein, and (2) an activation domain fused to a second protein. The DNA-binding domain targets the first hybrid protein to the UAS of the reporter gene; however, because most proteins lack an activation domain, this DNA-binding hybrid protein does not activate transcription of the reporter gene. The second hybrid protein, which contains the activation domain, cannot by itself activate expression of the reporter gene because it does not bind the UAS. However, when both hybrid proteins are present, the noncovalent interaction of the first and second proteins tethers the activation domain to the UAS, activating transcription of the reporter gene. When the virulence gene product (the first protein, for example) is already known to interact with another protein or nucleic acid, this assay can be used to detect agents that interfere with the binding interaction. Expression of the reporter gene is monitored as different test agents are added to the system; the presence of an inhibitory agent results in lack of a reporter signal.

When the function of the virulence gene product is unknown and no ligands are known to bind the gene product, the yeast two-hybrid assay can also be used to identify proteins that bind to the gene product. In an assay to identify proteins that bind to the first protein (the target protein), a large number of hybrid genes each encoding different second proteins are produced and screened in the assay. Typically, the second protein is encoded by a pool of plasmids in which total cDNA or genomic DNA is ligated to the activation domain. This system is applicable to a wide variety of proteins, and it is not even necessary to know the identity or function of the second binding protein. The system is highly sensitive and can detect interactions not revealed by other methods; even transient interactions may trigger transcription to produce a stable mRNA that can be repeatedly translated to yield the reporter protein.

Other assays may be used to search for agents that bind to the target protein. One such screening method to identify direct binding of test ligands to a target protein

is described in U.S. Patent No. 5,585,277, incorporated herein by reference. This method relies on the principle that proteins generally exist as a mixture of folded and unfolded states, and continually alternate between the two states. When a test ligand binds to the folded form of a target protein (i.e., when the test ligand is a ligand of the target protein), the target protein molecule bound by the ligand remains in its folded state. Thus, the folded target protein is present to a greater extent in the presence of a test ligand which binds the target protein, than in the absence of a ligand. Binding of the ligand to the target protein can be determined by any method which distinguishes between the folded and unfolded states of the target protein. The function of the target protein need not be known in order for this assay to be performed. Virtually any agent can be assessed by this method as a test ligand, including, but not limited to, metals, polypeptides, proteins, lipids, polysaccharides, polynucleotides and small organic molecules.

Another method for identifying ligands for a target protein is described in Wieboldt *et al.*, *Anal. Chem.*, *69*:1683-1691 (1997), incorporated herein by reference. This technique screens combinatorial libraries of 20-30 agents at a time in solution phase for binding to the target protein. Agents that bind to the target protein are separated from other library components by centrifugal ultrafiltration. The specifically selected molecules that are retained on the filter are subsequently liberated from the target protein and analyzed by HPLC and pneumatically assisted electrospray (ion spray) ionization mass spectroscopy. This procedure selects library components with the greatest affinity for the target protein, and is particularly useful for small molecule libraries.

The inhibitors/binders identified by the initial screens are evaluated for their effect on virulence in *in vivo* mouse models of streptococcal infections. Models of bacteremia, endocarditis, septic arthritis, soft tissue abscess, or pneumonia may be utilized. Models involving use of other animals are also comprehended by the invention. For example, rabbits can be challenged with a wild type streptococcal strain before or after administration of varying amounts of a putative inhibitor/binder compound. Control animals, administered only saline instead of putative inhibitor/binder compound provide a standard by which deterioration of the test animal can be determined. Other animal models include those described in the Animal and Plant Health Inspection Sevice, USDA, January 1, 1994 Edition,

§§113.69-113.70; Panciera and Corstvet, *Am. J. Vet. Res. 45*:2532-2537; Ames, *et al., Can. J. Comp. Med. 49*:395-400 (1984); and Mukkur, *Infection and Immunity 18*:583-585 (1977). Inhibitors/binders that interfere with bacterial virulence are can prevent the establishment of an infection or reverse the outcome of an infection once it is established.

The present invention is illustrated by the following examples. Example 1 describes constructions of *S. suis* mutants. Example 2 relates to screening for *S. suis* mutants. Example 3 addresses methods used to identify the copy number for signature tags. Example 4 describes evaluation of candidate mutants for virulence in mice. Example 5 addresses identification of genes related to virulence. Example 6 describes the murine efficacy model. Example 7 addresses screening for virulence in pigs. Example 8 relates to porcine vaccine efficacy studies. The Examples make reference to primer sequences. The sequences of primers used are included below as Table 2.

Table 2. Sequences of oligonucleotide primers

*Indicates oligo labeled with biotin at 5' end

SEQ ID NO:	Oligo Name	Sequence (5'-3')
25	TEF-326	CATGAATTCCATGGTACCCATTCTAAC
26	TEF-327	CTAGAATTCCTAGGTACCTACAACCTC
27	TEF-498	*CATGAATTCCATGGTACCCATTCTAAC
28	TEF-597	GGCCACGCGTCGACTAGTACNNNNNNNNNNCATAT
29	TEF-598	GGCCACGCGTCGACTAGTAC
30	TEF-599	GGCCACGCGTCGACTAGTACNNNNNNNNNNACGCC
31	TEF-663	GGCCACGCGTCGACTAGTACNNNNNNNNNNATGCAT
32	TEF-664	GGCCACGCGTCGACTAGTACNNNNNNNNNNGTTAAC
33	TEF-665	GGCCACGCGTCGACTAGTACNNNNNNNNNNTTCGAA
34	TEF-666	GGCCACGCGTCGACTAGTACNNNNNNNNNNGATATC
35	TEF-667	GGCCACGCGTCGACTAGTACNNNNNNNNNNTGATCA
36	TEF-668	GGCCACGCGTCGACTAGTACNNNNNNNNNNCAGCTG
37	TEF-705	AGGAGTTCTTGAATTTCACGATAGC
38	TEF-708	CGATTAAGGGCATGGAAACAATTCG
39	DEL-2121	AAAGCGCCCTCTATTGGTTCTGC
40	DEL-2126	GGTTTGATGTTGCGATTAATAGC
41	DEL-1235	GAAGGAGAAAACTCACCGAGGCA
42	DEL-1318	AGCACATCATCATCATAAGGTTT
43	DEL-1462	TCATTCACTACCAATTTGGGATG
44	DEL-2403	CCGAAGAAATGGAACGCTCT
45	DEL-2456	CAGCGAGGATATATCAATGAAC

N = A,G,C or T

Example 1. Construction of *S. suis* Library Containing Transposon-Tagged Mutants

A library of *Streptococcus suis* serotype 2 #R735 (reference strain) for signature tagged-mutants was constructed using the suicide vector pGh9:ISS1 [Maguin, *et al., J Bacteriol. 178:931-935* (1996)]. The ISS1 elements of the vector allow integration of the plasmid vector into the chromosome while encoding for erythromycin resistance. Moreover, the replicon of pGh9:ISS1 is thermosensitive that permits replication at 28°C but is lost at 37°C. Plasmid pGh9:ISS1 was modified to include sequence tags that contained a semi-random $[NK]_{35}$ sequence corresponding to the tags used in "Antibacterial Vaccine Applications" (US20040110268 A1). Briefly, sequence tags were PCR amplified using TEF326-327, digested with *Eco*RI

and ligated into the *Eco*RI site of pGh9:ISS1. These modified plasmids were used for the mutagenesis of *S. suis* as described below.

Electroporation-competent *S. suis* serotype 2 #R735 cells were prepared essentially as described by Takamatsu, *et al.* ("Construction and Characterization of *Streptococcus suis-Escherichia coli* Shuttle Cloning Vectors." *Plasmid.* 45:101-113 2001) with minor modifications. Briefly, freshly grown *S. suis* serotype 2 #R735 wild-type colonies were used to inoculate 100 mls Todd Hewitt Yeast Extract broth (THY). The initial OD_{600} of this culture, measured with a 300 mls side arm flask and a Bausch and Lomb Spectronic 70, was below 0.1. The broth culture was incubated at 37°C with shaking until the OD_{600} was between 0.3 and 0.5, and then distributed into two pre-chilled, sterile, 50 ml Corning tubes. While at 4°C, the cells were centrifuged at 4600 rpm for 10 minutes in an Eppendorf Centrifuge 5804 R. The cells were washed with 25 mls of pre-chilled CTB (Chemical Transformation Buffer, 55 mM $MnCl_2$, 15 mM $CaCl_2$, 250 mM KCl, and 10 mM PIPES [piperazine-*N*,*n*'-bis(2-ethanesulfonic acid) pH 6.7]), resuspended in 25 mls of pre-chilled CTB, and incubated on ice for 30 minutes. The cells were again centrifuged at 4600 rpm for 10 minutes at 4°C. The pellet was washed twice with 25 mls of pre-chilled EB (Electroporation Buffer, 0.3 M sucrose and 2 mM K_2HPO_4, pH 8.4). The cells were resuspended in 1 ml of pre-chilled EB containing 15% glycerol. Aliquots of 100 µl were immediately distributed into microcentrifuge tubes in a dry ice/ethanol bath. Electroporation-competent cells were stored at -70°C.

S. suis serotype 2 #R735 competent cells were electroporated with pGh9:ISS1 plasmids containing sequence tags as described as follows. Frozen 100 µl aliquots of cells were thawed on ice and combined with 5 µl plasmid DNA in pre-chilled 0.2 cm sterile electroporation cuvette. The cells and DNA mixture was pulsed immediately at 2.5 kV, 200Ω, and 25µF. The mixture was diluted with 400 µl THY broth containing 10% sucrose and 10 mM $MgCl_2$. The broth culture was incubated at 28°C for 2 hours with shaking. The entire 500 µl solution was spread on THY agar containing 0.5mg/ml erythromycin and incubated at 28°C with 5% CO_2 for 48 hours. Electroporations were performed for every pGh9:ISS1 plasmid containing different sequence tags. This resulted in 87 different *S. suis* serotype 2 #R735 strains, each containing a different sequence tag. A 96-well master plate was created with glycerol

cultures and stored at -70°C.

The presence of pGh9:ISS1 within erythromycin-resistant colonies was verified by PCR. Template for PCR consisted of 1 µl from a suspension of one bacterial colony in 60 µl of sterile, ddH_2O. Primers used were DEL2121 and DEL2126. The concentrations of PCR reagents per reaction were as follows: 1X XL Buffer II, 0.8 mM dNTP blend, 1.5 mM $Mg(OAc)_2$, 0.5 µM DEL2121, 0.5 µM DEL2126, and 1 Unit r*Tth* DNA Polymerase. Thermocycling was performed on an Applied Biosystems GeneAmp PCR System 2700 as follows: 94°C for 2 minutes, 30 cycles of (95°C for 30 seconds, 55°C for 1 minute, 72°C for 1.5 minutes), 72°C for 10 minutes, and held at 4°C. Confirmation of pGh9:ISS1 resulted from a PCR product of approximately 1 kb as determined by agarose gel electrophoresis.

The pGh9:ISS1 plasmids were forced to integrate into the *S. suis* chromosomal DNA. This was accomplished by growing overnight broth cultures, each culture containing *S. suis* serotype 2 #R735 with a different sequence tag inserted into pGh9:ISS1. The broth cultures were diluted 1000-fold (100 µl total volume in a 96-well plate), plated onto BHI (Brain Heart Infusion) agar plates containing 0.5mg/ml erythromycin ($Em^{0.5}$), and incubated overnight at 37°C with 5% CO_2. The resultant plates contained several hundred CFU (colony-forming units). Pools were constructed by inoculating 100 µl BHI/$Em^{0.5}$ broth in 96-well plates. Well A1 of pool one was inoculated with a colony of erythromycin-resistant *S. suis* serotype 2 #R735 containing pGh9:ISS1 with sequence tag one using a sterile toothpick. All of the wells of the plate were inoculated with the strain containing the appropriate sequence tag, until the entire plate was inoculated. This was continued for 30 plates, thus, creating 30 pools with 87 different *S. suis* serotype 2 #R735 signature-tagged mutants in each pool.

Example 2. Murine Screening of S. suis Serotype 2 #R735 STM Pools

Thirty pools were screened through murine models to identify attenuated candidates. Frozen pools of *Streptococcus suis* serotype 2 #R735 signature tagged mutants were removed from -70°C storage and subcultured, using a sterile 96-nail stamp, to a new 96-well round bottom plate (Corning Costar; Cambridge, MA) containing 200 µl of Todd Hewitt broth (Becton Dickinson; Cockeysville, MD) with 0.5mg/ml erythromycin (TH/$Em^{0.5}$) per well. Plates were incubated without shaking

overnight at 37°C with 5% CO_2. All 200 µl from each well of one plate was combined into a 50 ml, sterile Falcon tube and vortexed. One ml of this pooled solution was combined with 49 mls of sterile TH/$Em^{0.5}$. The cells from the remaining pooled solution were pelleted and stored at 4°C. The pelleted cells were used as the source of input pool total DNA. The diluted, pooled solution was incubated at 37°C with 5% CO_2 while shaking at 100 rpm. At an OD_{550} of approximately 0.05, CF-1 mice were infected with 1 ml of culture (approximately 1 x 10^7 CFU/ml) by intraperitoneal administration. Five mice were infected for each pool. After 24 hours post-infection, the mice were sacrificed and spleens harvested. The five spleens infected with the same pool were combined, homogenized, and plated on TH/$Em^{0.5}$ agar plates. Plates were incubated at 37°C with 5% CO_2 overnight. The following day, 10 mls of TH/$Em^{0.5}$ broth was added to the surface of the plates. The resulting colonies were gently scraped from the surface of the plate and homogenized by repeat pipetting. A 700 µl aliquot of this was used as the source of recovery pool total DNA.

Genomic DNA from the STM input and recovery pools was isolated using traditional or commercial techniques. For both techniques, 700 µl of cells was combined with 67µl of lysozyme (at 42mg/ml) and incubated at 37°C for 30 minutes. DNA was isolated from this solution by either phenol:chloroform extraction or by utilizing the Wizard Genomic DNA Purfication Kit (Promega; Madison, WI).

Example 3. Multiplexed Quantification of Genetic Sequences from Pools of Genomic DNA

The quantification of the copy number for every signature tag in each pool was determined utilizing microsphere fluorescence and fluidics with the Luminex xMAP technology (Luminex; Austin TX). Since each microsphere is specific for one oligonucleotide sequence, the relative amount of every signature tag in each pool was determined. To prepare the assay, the sequence of the signature tag for all 96 pGH9:ISS1 plasmid was determined. All templates were sequenced with the BigDye™ Dye Terminator v. 3.0 Chemistry kit from PE Applied Biosystems (Foster City, CA, U.S.A.) and cleaned using either the Performa ® DTR 96-well standard plate kit (Edge BioSystems; Gaithersburg, MD) or CentriSep Spin Columns (Princeton Separations; Adelphia, NJ). Samples were run on an ABI Prism 377 or ABI 3700 DNA Sequencer. Sequencher 3.0 software (Genecodes, Corp.; Ann Arbor, MI, U.S.A.) was used to assemble and analyze sequence data.

Oligonucleotides (oligos STM01 through STM96) , 20 bp in length and modified at the 5' end with an amino-linker (AC_{12}), were synthesized by Sigma-Genosys Biotechnologies (The Woodlands, TX, U.S.A.) and are shown in Table 3. These oligomers are complementary to the DNA sequence tags in the pGH9:ISS1 transposon arrays.

Table 3. Sequences of Oligonucleotide Probes.

Oligo Name	Sequence (5' to 3')	SEQ ID NO
STM-01	TGGTGAGAGTGACCGCTAGG	SEQ ID NO:46
STM-02	GGATGTAGCGGTCTTTCTGG	SEQ ID NO:47
STM-03	GGCGATGTATGGCTAGGTTT	SEQ ID NO:48
STM-04	GTGGGGCTCTGTGTTTGGTG	SEQ ID NO:49
STM-05	GGTGGGGTGTCGGGGTGGGG	SEQ ID NO:50
STM-06	GTTGCGGTATCGCTATTTGT	SEQ ID NO:51
STM-07	TGGCGGGGTTGCGAGTTCTA	SEQ ID NO:52
STM-08	GGTTTAGCGATTTTGGGGGA	SEQ ID NO:53
STM-09	GGAGCTGGGTGTATAGATGG	SEQ ID NO:54
STM-10	AGGGGGTGTGGTGTGGGGAT	SEQ ID NO:55
STM-11	AGTGATGGGTCGGGGTTGTG	SEQ ID NO:56
STM-12	GGCGCTAGTAGGTTTGGTGT	SEQ ID NO:57
STM-13	TGTGCGATGGGTTGTTGGGG	SEQ ID NO:58
STM-14	ATGGGGCGGTCGGGATATGT	SEQ ID NO:59
STM-15	TGGGGTTATAGTGATTTTGG	SEQ ID NO:60
STM-16	GTATATGGAGAGCTGTGTAG	SEQ ID NO:61
STM-17	GTGGCATGTTTGTGTGAGGG	SEQ ID NO:62
STM-18	GGGGTGCGGTTTGGTTATTG	SEQ ID NO:63
STM-19	AGGTGCGAGCTCGGTCTGTA	SEQ ID NO:64
STM-20	ATCTCGGGGTCGGGGGATGG	SEQ ID NO:65
STM-21	GCGTGGTGTGCGGTCTATTT	SEQ ID NO:66
STM-22	TGTGGTTGGTGTGTAGAGTG	SEQ ID NO:67
STM-23	CGTGTGTGGTGTTTGGGGGT	SEQ ID NO:68
STM-24	GTGGGTGATGTGTGGATGGC	SEQ ID NO:69
STM-25	AGTGGGTTGTTGTGTCGAGA	SEQ ID NO:70
STM-26	GGTCGCGGGTGTTTCTGGGG	SEQ ID NO:71
STM-27	GGCGGGGTTGTTAGGGGTCG	SEQ ID NO:72
STM-28	GGTGGGCGCGGGGGCTGGGG	SEQ ID NO:73
STM-29	GGAGGGTTTGTTTGGGCGTT	SEQ ID NO:74
STM-30	ATCGGGGGAGGGGTTGCGCT	SEQ ID NO:75
STM-31	CGGGGTGTGGGGATAGTGGT	SEQ ID NO:76
STM-32	CTTTGGAGGGATGTTTTGAG	SEQ ID NO:77
STM-33	CTCTTTGCGCTTTTTAGTGG	SEQ ID NO:78
STM-34	TGGAGTGGTATGGCTCGCGG	SEQ ID NO:79
STM-35	TAGTTGCTGGTGGTTTGTGT	SEQ ID NO:80
STM-36	GTGCGCTCGCGATCGATGTC	SEQ ID NO:81
STM-37	AGGGCTTGGGATTGGGAGTG	SEQ ID NO:82
STM-38	GGATGTAGCGGTCTTTCTGG	SEQ ID NO:83
STM-39	CGGGTGTGGTCGCTCTGTTT	SEQ ID NO:84
STM-40	GGTGGGCGCGGGGGCTGGGG	SEQ ID NO:85

STM-41	CGTGGGGTGGTGGGCGATTT	SEQ ID NO:86
STM-42	TGCGCGAGTGGGTTAGTGGG	SEQ ID NO:87
STM-43	AGGTCTCGAGATGTCTGTCT	SEQ ID NO:88
STM-44	AGTGCGCGGGTTTTGGGTGT	SEQ ID NO:89
STM-45	ATGGGTGAGGGAGTGAAGAG	SEQ ID NO:90
STM-46	GGTGAGTGGTAGAGGGGTCG	SEQ ID NO:91
STM-47	GGTGTGTTCCGGGTCGTGTT	SEQ ID NO:92
STM-48	AGAGTGTGCTTTGATGTGTG	SEQ ID NO:93
STM-49	GTATCGGTCGCGGGAATAGC	SEQ ID NO:94
STM-50	AGAGCGTGAGTTCTTTCGTG	SEQ ID NO:95
STM-51	ATGGATGGCGCGGTAGGTTG	SEQ ID NO:96
STM-52	GTGGAGGGTTCGGTTGTTCG	SEQ ID NO:97
STM-53	GGGTATGTTGGGGTAGAGGA	SEQ ID NO:98
STM-54	GGTCGATTCTCGTGGGTGAT	SEQ ID NO:99
STM-55	GGTGGGAGCGTTTGCGGGCG	SEQ ID NO:100
STM-56	GGCTTTAGTTATGGGGAGTG	SEQ ID NO:101
STM-57	GGGGTGCTGGCTGTCTGGTT	SEQ ID NO:102
STM-58	GGGTTTTGTGGGGTGTGGCG	SEQ ID NO:103
STM-59	TGGCGCGGGGTGTTGTTTTC	SEQ ID NO:104
STM-60	GGGTTGGTAGGGTTGGGTCT	SEQ ID NO:105
STM-61	GTGATGGGTGCGATCAGTGC	SEQ ID NO:106
STM-62	AGTGGTGTTTAGCGAGGTAT	SEQ ID NO:107
STM-63	ATGGGGATTTCGTGTTTTGG	SEQ ID NO:108
STM-64	TGGGCGGTGTCTAGATAGAG	SEQ ID NO:109
STM-65	AGGGTGGTTGGGCGAGGGCT	SEQ ID NO:110
STM-66	GGGGTGAGTGATGGTGGTCG	SEQ ID NO:111
STM-67	GCTTGGGCGGGCTATAGGGC	SEQ ID NO:112
STM-68	GAGCTGGTGGGTTGGGGTTT	SEQ ID NO:113
STM-69	GGGAGTTTGGGCGCTGGGTT	SEQ ID NO:114
STM-70	CGTGATGTTGGGAGGGTTAG	SEQ ID NO:115
STM-71	GGCTTTGGTTGGTGGTGGAG	SEQ ID NO:116
STM-72	GTATTTGTCTGGCTGTGTTG	SEQ ID NO:117
STM-73	AGAGGGCGTGCGTGAGTGGA	SEQ ID NO:118
STM-74	GGAGCGGGTTAGGGCGGTGT	SEQ ID NO:119
STM-75	TGTGTGGATCTCTCGGTGCT	SEQ ID NO:120
STM-76	TTTGTTATGGGGCTGCGTGA	SEQ ID NO:121
STM-77	CGGGAGTGGGATGGGTTTTG	SEQ ID NO:122
STM-78	GATTGTGGTAGGTGGCGGTA	SEQ ID NO:123
STM-79	GGGTGTGTATTTGGCTTGGG	SEQ ID NO:124
STM-80	GGGCGGGTTAGATATTGGGA	SEQ ID NO:125
STM-81	GCTCGCTTGGCGCTATGGGG	SEQ ID NO:126
STM-82	GGTGGAGTGTGGTCGGTCTT	SEQ ID NO:127
STM-83	ATGTAGTGTTATGGCTGGAT	SEQ ID NO:128
STM-84	CGGTCGCGTGATATGGTGTT	SEQ ID NO:129
STM-85	TGTTGGTGGAGCTTTTGATT	SEQ ID NO:130
STM-86	GGGTATCGCTGGGGAGATTG	SEQ ID NO:131
STM-87	GGTTCGAGTTGTTGAGCTTG	SEQ ID NO:132
STM-88	ATCGGGCTGGTGATAGATGG	SEQ ID NO:133
STM-89	AGTTGGGTCGGGAGCTTGCG	SEQ ID NO:134
STM-90	GGATTGATTGCTGGGTGGCG	SEQ ID NO:135

STM-91	GGGTGGGTGTCTGGCTGGGA	SEQ ID NO:136
STM-92	CTGTGTGGGGTTGTCTCTCG	SEQ ID NO:137
STM-93	TGGGTCTGGTTGTCGGGTTA	SEQ ID NO:138
STM-94	TTAGTTGCTCGTTTGGTGTA	SEQ ID NO:139
STM-95	CTCGTTATTTTAGGTTGGTC	SEQ ID NO:140
STM-96	TTGAGGTATGGTTAGATGTG	SEQ ID NO:141

Synthetic oligonucleotides complimentary to 20 bp of the 96 STM tags were covalently coupled to carboxylated microspheres as recommended by the manufacturer. Each bead was coupled to the corresponding oligonucleotide (bead set 01 coupled to oligonucleotide 01, etc.). A fresh aliquot of -20°C, desiccated EDC powder was brought to room temperature. The amine-modified oligo was resuspended in ddH_2O for a final concentration of 0.1 mM (0.1nmole/µl). The microspheres were resuspended according to manufacturer's recommendations. An aliquot of $5.0x10^6$ microspheres was transferred to a sterile microcentrifuge tube and pelleted by centrifugation at 8000 x g for 1 minute. The supernatant was carefully discarded and the microspheres were resuspended in 50 µl 0.1 M MES by vortexing and sonication. 0.2 nmole of amine-modified oligo was added to the microsphere solution and then vortexed. A fresh aliquot of 10 mg/ml EDC in ddH_2O was prepared, and 2.5 µl of fresh 10 mg/ml EDC was immediately added to the microspheres and then vortexed. The microsphere/oligonucleotide solution was incubated for 30 minutes at room temperature in the dark. A second solution of 10 mg/ml EDC was prepared, and another 2.5 µl aliquot of fresh EDC was added to the microspheres. The solution was vortexed and again incubated 30 minutes at room temperature in the dark. After the final incubation, the microspheres were washed with 1.0 ml of 0.02% Tween-20, followed by a second wash step with 1.0 ml of 0.1% SDS. The microspheres were finally resuspended in 100 µl TE, pH 8.0, and stored at 2-8°C in the dark.

The genomic DNA isolated from the STM pools was used as a template in PCR for the amplification of the sequence tags. Primers used for amplification annealed to common sequences flanking the unique sequence tags in all mutants. The primers used were TEF-327 and biotinylated TEF-498. The concentrations of PCR reagents per reaction were as follows: 1X XL Buffer II, 0.8mM dNTP blend, 1.5mM $Mg(OAc)_2$, 0.5µM TEF327, 0.5µM TEF498, and 1 Unit r*Tth* DNA Polymerase. The

reaction conditions were as follows: 94°C for 2 minutes, 30 cycles of (95°C for 30 seconds, 55°C for 1 minute, 72°C for 1.5 minutes), 72°C for 10 minutes, and held at 4°C.

The amplified sequence tags were then hybridized to the appropriate microspheres. At all times, care was taken to assure minimized exposure of the microspheres to light. Microspheres previously coupled with amine-modified oligonucleotides were resuspended by vortexing for 30 seconds and sonication for 1 minute. A 1.0 ml Working Microsphere Mixture was created by diluting the coupled microsphere stocks in 1.5X TMAC Hybridization Buffer, to a final concentration for each bead set of 150 microspheres/µl. The Working Microsphere Mixture was vortexed for 30 seconds and sonicated for 1 minute. Aliquots of 33 µl of the Working Microsphere Mixture (containing approximately 5000 microspheres of each set) were added to each sample well or background well of a 96-well PCR plate. Five microliters of biotinylated PCR product was added to each sample well; 5 µl of TE buffer, pH 8.0, was added to the background well. The volume of the final solution in the well was brought up to 17 µl with TE buffer, pH 8.0. The samples were gently mixed by repeated pipetting. The plate was sealed with aluminum tape to prevent evaporation and light exposure. Using an Applied Biosystems GeneAmp PCR System 2700, the plate was incubated under the following conditions: 10 minutes at 95°C, followed by a hold at 45°C for a minimum of 15 minutes. The microspheres were pelleted by centrifugation at 2,500 x rpm in a Beckman GS-6R centrifuge for 10 minutes, and 25 µl of the supernatant was carefully removed with a pipette. For each reaction, 75 µl of fresh reporter mix, and 4 ng/µl of streptavidin, R-phycoerythrin in 1X TMAC hybridization buffer, was added to each well. The samples were gently mixed by repeated pipetting, and transferred to a 96-well plate designed to fit onto the Luminex XYP platform. With the XYP platform of the Luminex xMAP™ system held at 45°C, the samples were analyzed according to the manufacturer's recommendations.

The input and recovery DNA from 30 pools was analyzed for possible attenuated mutants using the Luminex xMAP™ system. If the growth of a mutant was inhibited in the murine host, the recovered signal was lower compared to the input signal, and resulted in a high input:recovered ratio.

Example 4. Evaluation of Candidate Mutants for Virulence

Luminex analysis resulted in 97 potentially attenuated mutants that exhibited reduced growth in the recovery pool relative to the input pool. These mutants were isolated from frozen stock of original cultures and analyzed individually to verify attenuation. Individual candidate mutants were grown overnight in 400 µl of TH/$Em^{0.5}$ broth at 37°C with 5% CO_2. The cultures were refreshed into 4 mls of TH/$Em^{0.5}$ and incubated at 37°C with 5% CO_2 while shaking at 100 rpm until the OD_{550} reached approximately 0.7. Three CF-1 mice per mutant were infected, each with 1 ml of culture by intraperitoneal administration. Attenuation was determined by comparing mortality and health after 72 hours relative to the wild-type positive control and TH/$Em^{0.5}$ negative control.

Results indicated that 58 of 97 potential transposon mutants produced some level of attenuation, having at least 1 out of 3 mice surviving compared to the wild-type, where 0 of 3 mice survived.

Example 5. Identification of Genes Tagged by Transposon Required for Virulence

The identity of the open reading frames disrupted by the transposon was determined by arbitrary PCR or single primer PCR techniques. These techniques allowed for the amplification of DNA flanking the transposon insertional element. The arbitrary PCR procedure consisted of a protocol modified from that previously described by Rossbach *et al.* (Rossbach *et al., Environmental Microbiology. 2(4):373-382* 2000). Primary amplification of DNA left of the ISS1 insertional element was performed using TEF-705. Primary amplification of the DNA right of the ISS1 element was performed using TEF-708. For both amplifications, a mix of up to 8 arbitrary primers was used. These included TEF-597, TEF-599, TEF-663, TEF-664, TEF-665, TEF-666, TEF-667, TEF-668. The concentrations of PCR reagents per reaction were as described previously. Template consisted of 5 µl of 5 ml overnight broth culture, or up to 5µl of genomic DNA preparation. Thermocycling was performed as follows: 2 minutes at 94°C, 14 cycles of (15 seconds at 94°C, 30 seconds touchdown starting at 50°C, 5 minutes at 72°C), 30 cycles of 15 seconds at 94°C, 30 seconds at 50°C, 5 minutes at 72°C), 7 minutes at 72°C, and held at 4°C. Secondary amplification of the DNA left of the ISS1 element was performed using nested primer DEL-2122. Secondary amplification of the DNA right of the ISS1

element was performed using nested primer DEL-2403. For both amplifications, another nested primer, TEF-598, was used. The concentrations of PCR reagents per reaction were as described above. Template consisted of 1 µl of primary amplification product.

Single primer PCR was performed essentially as described by Karlyshev *et al.* (Karlyshev *et al., BioTechniques. 28:1078-1082* 2000), followed by an second round of amplification using nested primers to improve product concentration with limited background. As described above, primary amplification of DNA flanking the left or right of the ISS1 insertional element used primers TEF-705 or TEF-708, respectively. The concentrations of PCR reagents per reaction were as described previously. Template consisted of 5 µl of 5 ml overnight broth culture, or up to 5µl of genomic DNA preparation. Thermocycling was performed as follows: 1 minute at 94°C, 20 cycles of (30 seconds at 94°C, 30 seconds at 50°C, 3 minutes at 72°C), 30 cycles of (30 seconds at 94°C, 30 seconds at 30°C, 2 minutes at 72°C), 30 cycles of (30 seconds at 94°C, 30 seconds at 50°C, 2 minutes at 72°C), 7 min at 72°C, and held at 4°C. Secondary amplification of the DNA left of the ISS1 element was performed using nested primers TEF-705 and DEL-2122. Secondary amplification of the DNA right of the ISS1 element was performed using nested primers TEF-708 and DEL-2403. The concentrations of PCR reagents per reaction were as described previously. Template consisted of 1 µl of primary amplification product.

All PCR products were analyzed by electrophoresis of the entire reaction in a 1% agarose gel. Desired products were excised from the gel. DNA was recovered from the agarose slice via QIAquick® Gel Extraction Kit according to the manufacturer's recommendations (Qiagen Inc.). Sequencing reactions of the PCR products were performed using primers DEL-2122 or DEL-2403 as specified above. Table 2 details the individual mutants and the identity of the disrupted open reading frames.

Table 4. S. suis #R375 Attenuated* STM Mutants

*All STM strains below resulted in 3/3 or 2/3 mice surviving the attenuation study

Gene Identity	Gene Description	ORF targeted	S. suis #R735 STM Mutants
gtfA****	sucrose phosporylase	SEQ ID NO:1	22E1
guaB	inosine monophosphate dehydrogenase	SEQ ID NO:3	25F9
lin0523	sequence in *Listeria innocua*	SEQ ID NO:5	01F2
manM	putative mannose-specific phosphotransferase	SEQ ID NO:7	26C8
purA	adenylosuccinate synthetase	SEQ ID NO:9	27D12
purD	phosphoribosylamine-glycine ligase	SEQ ID NO:11	01D7
			20D7
			20F2
scrB	sucrose-6-phosphate hydrolase	SEQ ID NO:13	01F7
			13F2
scrR	sucrose regulon regulatory protein	SEQ ID NO:15	25E1
SMU.61	putative transcriptional regulator	SEQ ID NO:17	26F2
spr1018	Conserved hypothetical protein	SEQ ID NO:19	27F12
SpyM3-0908.	Putative amino acid ABC transporter	SEQ ID NO:21	07F2

treR***	probable transcriptional regulator	SEQ ID NO:23	10F2
SP0843	a putative deoxyribose-phosphate aldolase	SEQ ID NO: 142	04H1
EndoD	endo-beta-N-aceytlglucosaminidase	SEQ ID NO: 144	05H2
neuB	putative N-acetyl neuramic acid synthetase	SEQ ID NO: 146	06F2
pgdA	peptidoglycan GlcNAc deacetylase	SEQ ID NO: 148	11C8
glnQ	ABC transporter involved in glutamine transport, encoding the ATP-binding protein	SEQ ID NO: 150	13F7
nadR	transcriptional regulator	SEQ ID NO: 152	14D3

*** = No Hits using BLASTN of 200bp flanking ISS1, but definite match using BLASTN with entire ORF
****=Only 26aa of Arb PCR product match to fused-ccdB, but ORF match to gftA

SEQ ID NO:1 and 2

```
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 K  D  L  K  K  V  L  E  G  P  L  K  D  V  V  G  G  I  H  I
ctgccgtttttcccatcatcaggtgaccgtgggtttgcgccaatggattatacaaaggtg
 L  P  F  F  P  S  S  G  D  R  G  F  A  P  M  D  Y  T  K  V
gaccctgcatttggagattggtcagatattgaagcactaagcaaagactattacctcatg
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 F  D  F  M  I  N  H  I  S  A  K  S  P  Y  F  L  D  F  L  E
aagaaagatgaatcagcttatgcggatttgtttattcgttataaaaacttttggccaaat
 K  K  D  E  S  A  Y  A  D  L  F  I  R  Y  K  N  F  W  P  N
ggtgagccaacgcaagaagatgttgatttgatttataagcgtaaacctcgcgcaccttat
 G  E  P  T  Q  E  D  V  D  L  I  Y  K  R  K  P  R  A  P  Y
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SEQ ID NO:3 and 4

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SEQ ID NO:5 and 6

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SEQ ID NO:7 and 8

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SEQ ID NO:9 and 10

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 T  D  T  S  V  I  L  N  D  A  L  D  Q  G  K  R  V  L  F  E
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 G  A  Q  G  V  M  L  D  I  D  Q  G  T  Y  P  F  V  T  S  S
aacccagttgctggtggtgtgacgatcggtagcggtgtcggcccaagcaagattgacaag
 N  P  V  A  G  G  V  T  I  G  S  G  V  G  P  S  K  I  D  K
gttgttggtgtatgtaaggcctacactagccgtgttggtgacggaccatttccgactgaa
 V  V  G  V  C  K  A  Y  T  S  R  V  G  D  G  P  F  P  T  E
ttgcacgatgaaatcggagaccgtatccgcgaaatcggtaaagagtacggtacgacaact
 L  H  D  E  I  G  D  R  I  R  E  I  G  K  E  Y  G  T  T  T
ggccgtccacgccgtgtcggttggtttgactcagtggtgatgcgccatagccgccgtgtg
 G  R  P  R  R  V  G  W  F  D  S  V  V  M  R  H  S  R  R  V
tcaggtattaccaacttgtccctcaactcgattgacgtcttgtcaggtcttgggaccttg
 S  G  I  T  N  L  S  L  N  S  I  D  V  L  S  G  L  G  T  L
aaaatctgcgtggcttatgacttggatggtgagcgtattgaccactacccagcaagtttg
 K  I  C  V  A  Y  D  L  D  G  E  R  I  D  H  Y  P  A  S  L
gagcaactcaaacgttgcaaaccaatctacgaagaaatgccaggttggtctgaagacatc
 E  Q  L  K  R  C  K  P  I  Y  E  E  M  P  G  W  S  E  D  I
acaggtgtacgtagcctggatgaattgccagaagcggctcgcaactatgttcgtcgtatc
 T  G  V  R  S  L  D  E  L  P  E  A  A  R  N  Y  V  R  R  I
agcgaattggtaggcgttcgtatctcaaccttctcagtaggacctggtcgtgaacagacc
 S  E  L  V  G  V  R  I  S  T  F  S  V  G  P  G  R  E  Q  T
aacatccttgagagtgtatggagcgcaaaatag
 N  I  L  E  S  V  W  S  A  K  -
```

SEQ ID NO:11 and 12

```
atgaaacttttggttgtcgggtctggtggtcgtgaacatgctatcgcaaagaaattgtta
 M  K  L  L  V  V  G  S  G  G  R  E  H  A  I  A  K  K  L  L
gagtctgagcaggtagagcaggtctttgttgctcctggaaatgacggaatgactttagat
 E  S  E  Q  V  E  Q  V  F  V  A  P  G  N  D  G  M  T  L  D
ggtatcgagttagtcaatatcgggatctccgaacattctgctataatcaactttgctaag
 G  I  E  L  V  N  I  G  I  S  E  H  S  A  I  I  N  F  A  K
gaaaatgacgttgcttggacttttgtgggtccagacgatgctctggcagcaggaatcgtt
 E  N  D  V  A  W  T  F  V  G  P  D  D  A  L  A  A  G  I  V
gatgattttgaacaggcgggacttaaagcttttggccctagtcgtctagccgcggagcta
 D  D  F  E  Q  A  G  L  K  A  F  G  P  S  R  L  A  A  E  L
gagtggtcaaaagactttgccaaacaaatcatggtcaaatacggcattccaacagcagcc
 E  W  S  K  D  F  A  K  Q  I  M  V  K  Y  G  I  P  T  A  A
tttggcacattttccaacttcgaagaagccaaagcctacatcgaagagcagggtgcacca
 F  G  T  F  S  N  F  E  E  A  K  A  Y  I  E  E  Q  G  A  P
atcgtggtcaaggcggacggcttggcactgggcaagggcgtggtcgtggcggaaaccgtc
 I  V  V  K  A  D  G  L  A  L  G  K  G  V  V  V  A  E  T  V
gagcaggcggtcgaagcggcacgggagatgctcttggacaacaagttcggcgactcgggt
 E  Q  A  V  E  A  A  R  E  M  L  L  D  N  K  F  G  D  S  G
gcccgcgtggtcatcgaggagttcttggcgggtgaggagttttccctctttgctctggtc
 A  R  V  V  I  E  E  F  L  A  G  E  E  F  S  L  F  A  L  V
aatggcgaccaattttacattctgccgacagcccaggaccacaagcgtgcctttgacggc
 N  G  D  Q  F  Y  I  L  P  T  A  Q  D  H  K  R  A  F  D  G
gaccaaggtcccaacacaggtggtatgggggcttacgctcctgttccccacctgcctcaa
 D  Q  G  P  N  T  G  G  M  G  A  Y  A  P  V  P  H  L  P  Q
agcgtggtggacacagcggttgacaccattgtcaagccgattcttgagggcatgattgcg
 S  V  V  D  T  A  V  D  T  I  V  K  P  I  L  E  G  M  I  A
gacggccggtcttatctgggcgtgctctatgctggcttgattctgaccgaccaaggtccc
 D  G  R  S  Y  L  G  V  L  Y  A  G  L  I  L  T  D  Q  G  P
aaggtcatcgagttcaacgcccgttttggcgacccagaaacccagattatcctgcctcgc
 K  V  I  E  F  N  A  R  F  G  D  P  E  T  Q  I  I  L  P  R
ctgacctctgactttgctcagaacatcgacgacatcctccacaaacgcccgacacagctg
 L  T  S  D  F  A  Q  N  I  D  D  I  L  H  K  R  P  T  Q  L
acttggctggatagtggcgtgacgctgggtgttgtcgtggcatcaaacggctaccctctg
 T  W  L  D  S  G  V  T  L  G  V  V  V  A  S  N  G  Y  P  L
gactacgaaaaaggcgtagagttgccagcaaagaccgagggcgacatcacgacctactat
 D  Y  E  K  G  V  E  L  P  A  K  T  E  G  D  I  T  T  Y  Y
gcaggggctcgttttgcggaaaatagcagagcactgctttcaaacggcggtcgggtttat
 A  G  A  R  F  A  E  N  S  R  A  L  L  S  N  G  G  R  V  Y
atgctagtcaccacagcagacaccgtccaagacgcccaagaaaaaatttactcggagctg
 M  L  V  T  T  A  D  T  V  Q  D  A  Q  E  K  I  Y  S  E  L
aaaaatcaagataccacaggcctcttttatcggacagatattggaagtaaggcagtaaaa
 K  N  Q  D  T  T  G  L  F  Y  R  T  D  I  G  S  K  A  V  K
taa
```

SEQ ID NO:13 and 14

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atgtcaaacgtttatcacattttgtttcaattttatcaaaaaacattgttaaaacttgat
 M  S  N  V  Y  H  I  L  F  Q  F  Y  Q  K  T  L  L  K  L  D
ttttcgcaagaaaacgtttactatattaatgaaaaactagaaaatttggagaaaattatg
 F  S  Q  E  N  V  Y  Y  I  N  E  K  L  E  N  L  E  K  I  M
gcatttacaacaggggagcgttacagagcttatgctgactggacacccgaatacattgaa
 A  F  T  T  G  E  R  Y  R  A  Y  A  D  W  T  P  E  Y  I  E
aaaataaagaagaatacagagagttctccttggaggacaaatttccatattgaaacacca
 K  I  K  K  N  T  E  S  S  P  W  R  T  N  F  H  I  E  T  P
catggactgttaaatgatccaaatggattttcttattttaatggaaagtggactcttttt
 H  G  L  L  N  D  P  N  G  F  S  Y  F  N  G  K  W  T  L  F
taccaatacttcccattcggtgctgcgcacggattgaaatcgtgggtccatacagaatca
 Y  Q  Y  F  P  F  G  A  A  H  G  L  K  S  W  V  H  T  E  S
acagatttggtccattttgaagaaactggtacggttatgtacccagatacaccacttgat
 T  D  L  V  H  F  E  E  T  G  T  V  M  Y  P  D  T  P  L  D
agccatggtgcctactctggatctgccatggaatttggcgacaagctcttcctcttctac
 S  H  G  A  Y  S  G  S  A  M  E  F  G  D  K  L  F  L  F  Y
actggaaatgtccgcgatgaaaattgggttcgccatccataccaaatcggtgccttgatg
 T  G  N  V  R  D  E  N  W  V  R  H  P  Y  Q  I  G  A  L  M
gataaagatggtaagattgaaaagattgataaagtcttgattgagcaaccagaagatacc
 D  K  D  G  K  I  E  K  I  D  K  V  L  I  E  Q  P  E  D  T
accgaccatttccgtgatccacaaattttcaactataaagggcaatattacgctattgtc
 T  D  H  F  R  D  P  Q  I  F  N  Y  K  G  Q  Y  Y  A  I  V
ggtggacaaaatctggataaaaagggaattgtcaaactttataaagctgaaaataacgac
 G  G  Q  N  L  D  K  K  G  I  V  K  L  Y  K  A  E  N  N  D
tacctcaactggtcctatgttggtgaccttgattttgccaatgatatgaccgcttatatg
 Y  L  N  W  S  Y  V  G  D  L  D  F  A  N  D  M  T  A  Y  M
atggaatgcccaaatattgcgttcgtcggtgagcaacccatccttctctattgtccacaa
 M  E  C  P  N  I  A  F  V  G  E  Q  P  I  L  L  Y  C  P  Q
ggtttggacaaagaggtattggactatggcaatatctatccaaatatgtacaagattggt
 G  L  D  K  E  V  L  D  Y  G  N  I  Y  P  N  M  Y  K  I  G
cagagtttcgttcctgaaacagcaagcattgtcgaaccgagcgaattgatcaatctggac
 Q  S  F  V  P  E  T  A  S  I  V  E  P  S  E  L  I  N  L  D
tatggttttgagtgctatgcaacccaggcctttaatgcaccagacggtcgaactctatct
 Y  G  F  E  C  Y  A  T  Q  A  F  N  A  P  D  G  R  T  L  S
gttagctggttggcacttccagatgtggaatacccaaccgatgcttatgactaccaaggc
 V  S  W  L  A  L  P  D  V  E  Y  P  T  D  A  Y  D  Y  Q  G
tgtctgtcattggttaaagaattaaccatcaaggacggtaagctctaccaatatccagtc
 C  L  S  L  V  K  E  L  T  I  K  D  G  K  L  Y  Q  Y  P  V
gatgcgattaccagtcttcgtaaagaagctcagccattttcagcccaaaaagaaaccaat
 D  A  I  T  S  L  R  K  E  A  Q  P  F  S  A  Q  K  E  T  N
aatgtttacgaattggaacttgatttttcagcgagcacgaagcacgaaatcgtattgttc
 N  V  Y  E  L  E  L  D  F  S  A  S  T  K  H  E  I  V  L  F
gcaaatgaagcagaaaaaggcttggtcttgtctgtagacactgaacaaggccagattaca
 A  N  E  A  E  K  G  L  V  L  S  V  D  T  E  Q  G  Q  I  T
ttggaccgtggcaactgtggtcagccattcgcactcgatttcggcacaagtcgctcttgc
 L  D  R  G  N  C  G  Q  P  F  A  L  D  F  G  T  S  R  S  C
gagatttctccaggagctcttacagccaatatcttcatcgacaaatctgtctttgaaatc
 E  I  S  P  G  A  L  T  A  N  I  F  I  D  K  S  V  F  E  I
tttatcaataaaggagagaaagtattttctggtcgtgtcttccctgatgcggatcagtct
 F  I  N  K  G  E  K  V  F  S  G  R  V  F  P  D  A  D  Q  S
ggtatagtcattacgactggtaatccgaccggaacctactaccatttagattatggtcgc
 G  I  V  I  T  T  G  N  P  T  G  T  Y  Y  H  L  D  Y  G  R
aaaactaactga
 K  T  N  -
```

SEQ ID NO:15 and 16

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atggtcgcaaaactaactgatgtcgcacagttagctggggtcagcccaacaactgtctcg
 M  V  A  K  L  T  D  V  A  Q  L  A  G  V  S  P  T  T  V  S
cgcgttatcaataaaaaaggatacctatctgaaaaaaccattcgcaatgtcgaagaggct
 R  V  I  N  K  K  G  Y  L  S  E  K  T  I  R  N  V  E  E  A
atgcgtgaactaggctacaaacctaataatttagctcgtagtttgcaaggtaaatctgca
 M  R  E  L  G  Y  K  P  N  N  L  A  R  S  L  Q  G  K  S  A
aaattagttggtctcatcttcccaacaataaacaatatttttattctgaattgattagc
 K  L  V  G  L  I  F  P  T  I  N  N  I  F  Y  S  E  L  I  S
catttagaaaaagagcttttgatcgaggctataaaaccattatttgcaatagccagcac
 H  L  E  K  E  L  F  D  R  G  Y  K  T  I  I  C  N  S  Q  H
gagtccgataaggaacgtgagtatctagaaatgttagcagctaaccaagtagatgggatt
 E  S  D  K  E  R  E  Y  L  E  M  L  A  A  N  Q  V  D  G  I
atctcgggcagtcacaatctcggtatccaagactacgaccgtgtcgtagctccaattatc
 I  S  G  S  H  N  L  G  I  Q  D  Y  D  R  V  V  A  P  I  I
gcttttgaccgcaacctatctccttccattcccattgtctcttccgataattttgctggc
 A  F  D  R  N  L  S  P  S  I  P  I  V  S  S  D  N  F  A  G
ggggtattagctgcccaaaccttacaaaaagcgggatgtcacaatccattaatgattacg
 G  V  L  A  A  Q  T  L  Q  K  A  G  C  H  N  P  L  M  I  T
gggaacgatgattccaattcacctaccggccttcgccaagtaggttttacatctattttg
 G  N  D  D  S  N  S  P  T  G  L  R  Q  V  G  F  T  S  I  L
aacaaagccgaagtttttcatatctccagtgatttctcacctgtgcgtaaggagatggaa
 N  K  A  E  V  F  H  I  S  S  D  F  S  P  V  R  K  E  M  E
atccgagccattcttgaaaagcaaaaaccaaatggtatctttgtatctggcgatgcaacg
 I  R  A  I  L  E  K  Q  K  P  N  G  I  F  V  S  G  D  A  T
gctatgctggtatggaacgtcattcgttcaatgaaactgtctattccagaagatattaaa
 A  M  L  V  W  N  V  I  R  S  M  K  L  S  I  P  E  D  I  K
ttgattggttatgatggaaccagtttcatcgaaaactactaccctcaactgacaactatc
 L  I  G  Y  D  G  T  S  F  I  E  N  Y  Y  P  Q  L  T  T  I
aagcaaccattaaccgaaatagctcgcctgctggttgaactattagttgataaaatcgaa
 K  Q  P  L  T  E  I  A  R  L  L  V  E  L  L  V  D  K  I  E
ggaaaaaaactgacaaaaacagactatatcctgccgattagcctactagcaggagctagt
 G  K  K  L  T  K  T  D  Y  I  L  P  I  S  L  L  A  G  A  S
atataa
 I  -
```

SEQ ID NO:17 and 18

```
atgaacgataaggaatttggacagcgtgtacgtcaattacgagaatctgctagtatgaca
 M  N  D  K  E  F  G  Q  R  V  R  Q  L  R  E  S  A  S  M  T
cgtgaacagttttgtgacgatgaactggaactctctgtgcgccaattaactcgtattgaa
 R  E  Q  F  C  D  D  E  L  E  L  S  V  R  Q  L  T  R  I  E
gcaggtgcttccaagccgacttttttcaaagattcagtatattgcaactcgtttaggtatg
 A  G  A  S  K  P  T  F  S  K  I  Q  Y  I  A  T  R  L  G  M
ggactttacgagcttatgccagattatgtatctttacccgaaagatattccaagctgaag
 G  L  Y  E  L  M  P  D  Y  V  S  L  P  E  R  Y  S  K  L  K
tttgatgtgcttcgcaccccaacttatggtaatgaagatttggcggaaaagcgagatgcc
 F  D  V  L  R  T  P  T  Y  G  N  E  D  L  A  E  K  R  D  A
atgatgacagaaatctatgacgattattatgatgaattgcctgaggaggagaagatagca
 M  M  T  E  I  Y  D  D  Y  Y  D  E  L  P  E  E  E  K  I  A
atagatgcgattcaatcacgaattgatactttagagtcaggtacagcaggctttggaaaa
 I  D  A  I  Q  S  R  I  D  T  L  E  S  G  T  A  G  F  G  K
gagatactggaagactactttgaacaaatttttcgcaaacgaaagtatgaattgaatgat
 E  I  L  E  D  Y  F  E  Q  I  F  R  K  R  K  Y  E  L  N  D
ttgttgattgttaggctccatcttgaatatgttaggttatctagctgtgattcagaaata
 L  L  I  V  R  L  H  L  E  Y  V  R  L  S  S  C  D  S  E  I
tttagacagtttttgaaaattatagagcatttacatgagcaaatcaatatcatcaactca
 F  R  Q  F  L  K  I  I  E  H  L  H  E  Q  I  N  I  I  N  S
aatgatttatttgttttacgagacacgctattatcttgtgtaaatatattaggaagtaaa
 N  D  L  F  V  L  R  D  T  L  L  S  C  V  N  I  L  G  S  K
aaatattacgaaccaataccaaagatatttgacagtgtagataagattatacagtcgaca
 K  Y  Y  E  P  I  P  K  I  F  D  S  V  D  K  I  I  Q  S  T
caagattttcagaaaaagcccattgttagtgtattaaaatggaaatatgcacttttgtg
 Q  D  F  Q  K  K  P  I  V  S  V  L  K  W  K  Y  A  L  F  V
gataaggatcgggatgaggcagaaaagcattatctagatgcggtgctatttgcaaaattg
 D  K  D  R  D  E  A  E  K  H  Y  L  D  A  V  L  F  A  K  L
atagaaaatagagagttagaacagaagattgaagaagattggagagttgacaatcaataa
 I  E  N  R  E  L  E  Q  K  I  E  E  D  W  R  V  D  N  Q  -
```

SEQ ID NO:19 and 20

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atggttcgtttgaaaaatattttacttattttgcttggtgcagggctatttgcctttggg
 M  V  R  L  K  N  I  L  L  I  L  L  G  A  G  L  F  A  F  G
ctaaattatttaatcatgcccaatcgtctattcgagggtggcgcaactggattgacgctg
 L  N  Y  L  I  M  P  N  R  L  F  E  G  G  A  T  G  L  T  L
attatttactacctttttcatattcaaccttggataatgaacattgtgattaatattcca
 I  I  Y  Y  L  F  H  I  Q  P  W  I  M  N  I  V  I  N  I  P
ttgttcatcctcgggtggaaaatactcggaaagaagactctctatctcagtatcttagga
 L  F  I  L  G  W  K  I  L  G  K  K  T  L  Y  L  S  I  L  G
acctttagcgttacctttttggttggccatttttgaaaaaatccatttttcaatcaatcta
 T  F  S  V  T  F  W  L  A  I  F  E  K  I  H  F  S  I  N  L
caacaagatttgatactcgttagtatcctgggtgggattcttatgggactaggattagga
 Q  Q  D  L  I  L  V  S  I  L  G  G  I  L  M  G  L  G  L  G
accatcttccgtgctggcggaacgactgggggaagcgatattattgctcgtataggtcat
 T  I  F  R  A  G  G  T  T  G  G  S  D  I  I  A  R  I  G  H
aaattcctaccttactccattggacaaatcattcttgcagttgacatcctcatcctaact
 K  F  L  P  Y  S  I  G  Q  I  I  L  A  V  D  I  L  I  L  T
ctgattgttattgtttttaaagacctccgaacagttctctatactttaatgatggttgct
 L  I  V  I  V  F  K  D  L  R  T  V  L  Y  T  L  M  M  V  A
attgcttccaaggttattgattttgtcacggagggcggatatggtagcaagggtgttatg
 I  A  S  K  V  I  D  F  V  T  E  G  G  Y  G  S  K  G  V  M
attgtttctcaaaaatctgaccaattggctcaggctattgattgtgaaatcgagcgtggt
 I  V  S  Q  K  S  D  Q  L  A  Q  A  I  D  C  E  I  E  R  G
gtcacctttatcaaagcacaaggtttctacagcaaagccgatgtcaacatgatttactcg
 V  T  F  I  K  A  Q  G  F  Y  S  K  A  D  V  N  M  I  Y  S
gtcatatataagagccaactccaagagatgaaggagcttattcatcgtattgacccccac
 V  I  Y  K  S  Q  L  Q  E  M  K  E  L  I  H  R  I  D  P  H
gcatttatcaccattacagacgcacatgaagtactcggtgagggatttactttagatcga
 A  F  I  T  I  T  D  A  H  E  V  L  G  E  G  F  T  L  D  R
gataaaaaaccaatagaacgccattaa
 D  K  K  P  I  E  R  H  -
```

SEQ ID NO:21 and 22

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atgcgaggaacccccttacttttacaattaatttttgtttattacgtcttaccaagtgtg
 M  R  G  T  P  L  L  L  Q  L  I  F  V  Y  Y  Y  V  L  P  S  V
ggtattacatttgaccgaatgcctgctgtgattctggcctttacgctcaactatgcggcc
 G  I  T  F  D  R  M  P  A  V  I  L  A  F  T  L  N  Y  A  A
tatttctctgaaattttccgtggtggtattgaagctattccaaaaggtcagtatgaagct
 Y  F  S  E  I  F  R  G  G  I  E  A  I  P  K  G  Q  Y  E  A
gctaaggtattgaaatttacccctgttcagaccattcgctacattgtcttaccacaggtt
 A  K  V  L  K  F  T  P  V  Q  T  I  R  Y  I  V  L  P  Q  V
gtgaaaattgttctgccaagtgttttcaatgaagtcatgactttggtaaaggatacttct
 V  K  I  V  L  P  S  V  F  N  E  V  M  T  L  V  K  D  T  S
ttggtctatgcacttggggttagcgatttgttgttggctagtcgtactgcggccaatcgt
 L  V  Y  A  L  G  V  S  D  L  L  L  A  S  R  T  A  A  N  R
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SEQ ID NO:23 and 24

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```

Amino acid sequences for the *S. suis* genes were aligned with the homologous sequences identified in other streptococcal species. % identity and % similarity were calculated using BLASTP 2.2.6 [Tatusova TA and TL Madden, "BLAST 2 sequences- a new tool for comparing protein and nucleotide sequences." (1999) FEMS Microbiol Lett. **174:**247-250.], and are shown in Table 5.

Table 5. Homologues Identified in Other Streptococcal Species

S. suis	*S. agalactiae*			*S. pyogenes*			*S. pneumoniae*		
	Accession #	% Identity	% Similarity	Accession #	% Identity	% Similarity	Accession #	% Identity	% Similarity
GtfA	-	-	-				AAL00512	58	72
GuaA	AX608462	89	95	AX954530	89	95	AX571764	86	93
GuaB	AX606637	89	95	AX954534	89	95	AX194078	90	95
ManM	AX608227	76	86	CQ648284	76	85	CQ789120	77	84
PurA	AX602166	91	96	AX954533	91	96	AR219059	90	95
PurD	AX608096	83	91	AX953813	80	89	AR218851	85	91
ScrB	-	-	-	-	-	-	AX571764	70	81
ScrR	-	-	-	-	-	-	AR218855	72	85
SpyM3-0908	CQ655070	80	91	AX954530	80	91	AX571764	80	92

The attenuated mutants were further evaluated by Southern blot analysis to verify that the mutation resulted from a single insertion of the pGh9:ISS1 plasmid. DNA from each mutant was isolated as described previously and digested with the restriction enzyme *Hin*dIII (New England Biolabs; Beverly, MA) according to the manufacturer's suggested protocol. Digested DNA products were analyzed for completion of digestion via agarose electrophoresis and transferred to Hybond™-N^+ nucleic acid transfer membrane (Amersham Biosciences; Piscataway, NJ). Separately, a probe containing a 0.6 kb fragment of ISS1 was created by amplification of the DNA sequence from pGh9:ISS1 as follows. The concentrations of PCR reagents per reaction were as described previously. The primers used were DEL-2456 and DEL-2403. Template consisted of 1 µl of pGh9:ISS1 miniprep product. Thermocycling was performed as follows: 2 minutes at 94°C, 20 cycles of (30 seconds at 95°C, 1 minute at 55°C, 1.5 minutes at 72°C), 10 minutes at 72°C, and held at 4°C. The PCR product was analyzed via agarose gel electrophoresis, and a 0.6 kb band was excised from the gel. The DNA was eluted from the agarose slice using the QIAquick® Gel Extraction Kit according to the manufacturer's suggested protocol. The eluted 0.6 kb DNA product was DIG-labeled with the DIG-High Prime Kit according to the manufacturer's recommendations (Roche; Mannheim, Germany). The DIG-labeled ISS1 fragment probe was hybridized to the *Hin*dIII digested STM DNA and developed with colorimetric detection using NBT and BCIP. The resulting membrane was analyzed for determination of multiple insertions of pGh9:ISS1 into the *S. suis* serotype 2 #R735 STM mutants.

Example 6. Murine Efficacy Model

S. suis serotype 2 #R735 STM mutants that were shown to be attenuated in the murine model and had, by Southern blot confirmation, a single insertion of pGh9:ISS1 disrupting one open reading frame are to be further tested in mice for the ability to vaccinate against wild type. Briefly, approximately $1x10^8$ CFU per dose of each mutant will be used to vaccinate a group of CF-1 mice by intraperitoneal administration. A second vaccination may also be administered in the same manner. Approximately 3 weeks following the vaccination, mice will be challenged with $1x10^9$ CFU of the wildtype organism. After 7 days, mortality will be recorded and used to determine the vaccine efficacy.

Example 7. Porcine Screening of S. suis Serotype 2 #R735 STM Pools

The *S. suis* serotype 2 #R735 STM pools that were screened in mice will also be screened in a pig model. The frozen pools will be retrieved from -70°C storage and refreshed as described above. Intravenous administration of freshly grown culture, at $1x10^9$ CFU/dose, will be given to CDCD (cesarean-derived, colostrum-deprived) pigs. After 24 hours post-administration, the pigs will be euthanized. Attempts will be made to recover the *S. suis* serotype 2 #R735 STM mutants from the blood, liver, spleen, lung, brain, and joints. Tissues will be macerated, and the fluid from these tissues as well as fluid collected in the joints will be plated on $TH/Em^{0.5}$ agar plates, incubated overnight at 37°C with 5% CO_2 . DNA from recovered mutants will be isolated as described above. Molecular analysis of input and recovered DNA will be performed using the with the Luminex xMAP technology (Luminex; Austin TX) as described above.

Example 8. Porcine Screening of Individual *S. suis* Serotype 2 Attenuated Mutants

Potential attenuated mutants derived from the porcine screening of pools will be used to individually inoculate pigs either intravenously, intranasally, orally or intramuscularly. Mutants will be deemed attenuated if there is a reduction in mortality and/or clinical signs, as compared to inoculation with the wild-type *S. suis* organism.

Example 9. Porcine Vaccine Efficacy Studies

S. suis mutants that show a reduction in virulence, as described above, will be evaluated for their ability to stimulate immunity against further infection by wild-type

S. suis. Animals will be vaccinated once or twice with an appropriate amount of each mutant through a desired route, preferably intramuscularly. Subsequently animals will be challenged with a wild-type strain of *S. suis*, along with unvaccinated controls. Mutants that afford a reduction in mortality and/or clinical signs, as compared to unvaccinated controls, will be deemed efficacious.

Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

WHAT IS CLAIMED IS:

1. An attenuated streptococcal bacteria comprising a functional mutation of one or more virulence genes selected from the group consisting *of gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908, treR sp0843, endoD, neuB, pgdA, glnQ,* and *nadR*, wherein said functional mutation attenuates the bacteria.

2. The steptococcal bacteria of claim 1 wherein the functional mutation results in one or more of the following: decreased gene expression, decreased biological activity of the gene product, and / or, expression of an inactive gene product.

3. The steptococcal bacteria of claim 2 wherein the functional mutation results from one or more of the following: deletion of all or part of said gene, insertion in the gene, and / or one or more point mutations in the gene.

4. The streptococcal bacteria of claim 1 wherein said virulence gene comprises a polynucleotide selected from the group consisting of:

a) a polynucleotide sequence selected from group consisting of SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23,142,144,146,148,150, and 152; and

b) a polynucleotide that hybridizes to the complement of a polynucleotide sequence set forth in a) under conditions comprising a final wash in buffer comprising 2XSSC/0.1%SDS, at 35° C to 45° C; and

c.) a polynucleotide that encodes a polypeptide that has at least 70% identity and/or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153.

5. The streptococcal bacteria of claim 1 selected from the group consisting of *S. acidominimus, S. agalactiae, S. bovis, S. equinus, S. canis, S. cricetus, S. downei, S. dysgalactiae, S. entericus, S. equi, S. equinus, S. equisimilis, S. gallinaceus, S. iniae, S. mutans, S. parauberis, S. pluranimalium, S. porcinus, S. pyogenes, S. pneumoniae, S. sobrinus, S. uberis, S. suis,* and *S. zooepidemicus.*

6. The streptococcal bacteria of claim 1 that is *S. suis.*

7. An immunogenic composition which comprises a streptococcal bacteria comprising a functional mutation of one or more virulence genes selected from the group consisting of *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908, treR sp0843, endoD, neuB, pgdA, glnQ,* and *nadR*, wherein said functional mutation attenuates the bacteria.

8. The steptococcal bacteria of claim 7 wherein the functional mutation results in one or more of the following: decreased gene expression, decreased biological activity of the gene product; and / or expression of an inactive gene product.

9. The steptococcal bacteria of claim 7 wherein the functional mutation results from one or more of the following: deletion of all or part of said gene, an insertion in the gene, and / or one or more point mutations in the gene.

10. The streptococcal bacteria of claim 7 wherein said gene comprises a polynucleotide selected from the group consisting of:
a) a polynucleotide sequence selected from group consisting of SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23,142,144,146,148,150, and 152; and
b) a polynucleotide that hybridizes to the complement of a polynucleotide sequence set forth in a) under conditions comprising a final wash in buffer comprising 2XSSC/0.1%SDS, at 35° C to 45° C; and
c.) a polynucleotide that encodes a polypeptide that has at least 70% identity and or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153.

11. The streptococcal bacteria of claim 7 selected from the group consisting of *S. acidominimus, S. agalactiae, S. bovis, S. equinus, S. canis, S. cricetus, S. downei, S. dysgalactiae, S. entericus, S. equi, S. equinus, S. equisimilis, S. gallinaceus, S. iniae, S. mutans, S. parauberis, S. pluranimalium, S. porcinus, S. pyogenes, S. pneumoniae, S. sobrinus, S. uberis, S. suis,* and *S. zooepidemicus.*

12. A vaccine or an immunogenic composition of claims 7-11, which comprises a streptococcal bacteria comprising a functional mutation of one or more genes selected from the group consisting of *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908, treR, sp0843, endoD, neuB, pgdA, glnQ,* and *nadR,* wherein said functional mutation attenuates the bacteria.

13. The vaccine of claim 12 further comprising at least one of porcine reproductive and respiratory syndrome virus, pseudorabies virus, porcine circovirus type II, swine influenza virus, *Salmonella cholerasuis, Salmonella typhimurium, Erysipelothrix rhusiopathiae, Lawsonia intracellularis, Haemophilus parasuis, Bordetella bronchiseptica, Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Escherichia coli, Pasteurella multocida,* and *Clostridium perfringens* type A and type C.

14. A method for producing an attenuated streptococcal bacteria comprising the step of introducing a functional mutation into one or more genes selected from the group consisting of *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908 , treR, sp0843, endoD, neuB, pgdA, glnQ,* and *nadR,* wherein said functional mutation attenuates the bacteria.

15. The method of claim 14, wherein the functional mutation results in one or more of the following: decreased gene expression, decreased biological activity of the gene product, expression of an inactive gene product, or wherein the functional mutation results from deletion of all or part of said gene, or wherein the functional mutation results from an insertion in the gene, or wherein the functional mutation results from one or more point mutations in the gene or within regulatory sequences or genes..

16. The method of claim 14, wherein said gene comprises a polynucleotide selected from the group consisting of:

a) a polynucleotide sequence selected from group consisting of SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23, 142,144,146,148,150, and 152; and

b) a polynucleotide that hybridizes to the complement of a polynucleotide sequence set

forth in a) under conditions comprising a final wash in buffer comprising 2XSSC/0.1%SDS, at 35° C to 45° C; and

c.) a polynucleotide that encodes a polypeptide that has at least 70% identity and or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153.

17. The method of claim 16, wherein the bacteria is selected from the group consisting of *S. acidominimus, S. agalactiae, S. bovis, S. equinus, S. canis, S. cricetus, S. downei, S. dysgalactiae, S. entericus, S. equi, S. equinus, S. equisimilis, S. gallinaceus, S. iniae, S. mutans, S. parauberis, S. pluranimalium, S. porcinus, S. pyogenes, S. pneumoniae, S. sobrinus, S. uberis, S. suis,* and *S. zooepidemicus.*

18. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of :

(a) a polynucleotide sequence set forth in SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21, 23,142,144,146,148,150, and 152; and

(b) a polynucleotide that encodes a polypeptide that has at least 95% identity and or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs: 2, 4, 6, 8,1 0, 12, 14, 16, 18, 20, 22, 24, 143, 145, 147, 149, 151, and 153;

(c) a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NOs: SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22, 24,143,145,147,149,151, and 153;

(d) a polynucleotide which is the complement of any of (a), (b), (c) or (d).

19. The polynucleotide of claim 18 which is DNA.

20. A vector comprising the DNA of claim 19 wherein the vector is optionally selected from: an expression vector, or where the DNA is operatively linked to an expression control DNA sequence

21. A host cell stably transformed or transfected with the DNA of claim 20 inz a

manner allowing expression of the encoded polypeptide in said host cell.

22. A method for producing a recombinant polypeptide comprising culturing the host cell of claim 21 in a nutrient medium and isolating the encoded polypeptide from said host cell or said nutrient medium.

23. A isolated polypeptide produced by the method of claim 22.

24. An isolated polypeptide comprising a polypeptide that has at least 95%, 98% or complete identity and or similarity to a polypeptide selected from the group consisting of the polypeptide sequences set forth in SEQ ID NOs:2,4,6,8,10,12,14,16,18,20,22,24,143,145,147, 149,151, and 153.

25. An antibody, including a monoclonal antibody that is specifically reactive with the polypeptide of claim 24.

26. A method of using the monoclonal antibody of claim 25 for identifying a bacteria of claim 1 comprising the step of contacting said bacteria or an extract of said bacteria with said monoclonal antibody and the absence of binding of said monoclonal antibody.

27. A method of identifying an anti-bacterial agent comprising the steps of assaying potential agents for the ability to interfere with expression or activity of one or more streptococcal gene products selected from any gene products or polynucleotides from claims 1-24 the group consisting of *gtfA, guaB, lin0523, manM, purA, purD, scrB, scrR, SMU.61, spr1018, spyM3-0908 treR, sp0843, endoD, neuB, pgdA, glnQ,* and *nadR*,.

SEQUENCE LISTING

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Fuller, Troy E
Wilson, Thomas Larry
Martin, Stephen
Klein, Loretta K

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Asp Val Leu Leu Ile Pro Ala Glu Ser His Val Leu Pro His Asp Ile
20 25 30

Asp Leu Lys Thr Gln Leu Ala Pro Asn Leu Thr Leu Asn Leu Pro Ile
35 40 45

Ile Ser Ala Ala Met Asp Thr Val Thr Asp Ser Lys Met Ala Ile Ala
50 55 60

Met Ala Arg Ala Gly Gly Leu Gly Val Ile His Lys Asn Met Ser Ile
65 70 75 80

Ala Glu Gln Ala Asp Glu Val Arg Lys Val Lys Arg Ser Glu Asn Gly
85 90 95

Val Ile Ile Asp Pro Phe Phe Leu Thr Pro Glu His Thr Ile Ala Glu
100 105 110

Ala Glu Lys Leu Met Ala Thr Tyr Arg Ile Ser Gly Val Pro Ile Val
115 120 125

Glu Thr Leu Glu Asn Arg Lys Leu Val Gly Ile Ile Thr Asn Arg Asp
130 135 140

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Met Arg Phe Ile Ser Asp Tyr Ser Gln Pro Ile Ser Thr Asn Met Thr
145                 150                 155                 160

Ser Asp Glu Leu Val Thr Ala Pro Val Gly Thr Asp Leu Ala Thr Ala
                165                 170                 175

Glu Ala Ile Leu His Lys His Arg Ile Glu Lys Leu Pro Leu Val Asp
            180                 185                 190

Glu Asn Gly Arg Leu Ser Gly Leu Ile Thr Ile Lys Asp Ile Glu Lys
        195                 200                 205

Val Ile Glu Phe Pro Asn Ala Ala Lys Asp Glu Phe Gly Arg Leu Leu
    210                 215                 220

Val Ala Gly Ala Val Gly Val Thr Ser Asp Thr Phe Glu Arg Ala Glu
225                 230                 235                 240

Ala Leu Phe Glu Ala Gly Ala Asp Ala Ile Val Ile Asp Thr Ala His
                245                 250                 255

Gly His Ser Ala Gly Val Leu Arg Lys Ile Lys Glu Ile Arg Asp His
            260                 265                 270

Phe Pro Thr Arg Thr Leu Ile Ala Gly Asn Ile Ala Thr Ala Glu Gly
        275                 280                 285

Ala Arg Ala Leu Tyr Glu Ala Gly Val Asp Val Val Lys Val Gly Ile
    290                 295                 300

Gly Pro Gly Ser Ile Cys Thr Thr Arg Val Ile Ala Gly Val Gly Val
305                 310                 315                 320

Pro Gln Val Thr Ala Ile Tyr Asp Ala Ala Gly Val Ala Arg Glu Tyr
                325                 330                 335

Gly Lys Thr Ile Ile Ala Asp Gly Gly Ile Lys Tyr Ser Gly Asp Ile
            340                 345                 350

Val Lys Ala Leu Ala Ala Gly Gly His Ala Val Met Leu Gly Ser Met
        355                 360                 365

Phe Ala Gly Thr Asp Glu Ala Pro Gly Glu Thr Glu Ile Phe Gln Gly
    370                 375                 380
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Arg Lys Phe Lys Thr Tyr Arg Gly Met Gly Ser Ile Ala Ala Met Lys
385 390 395 400

Gln Gly Ser Lys Asp Arg Tyr Phe Gln Ala Ser Val Asn Glu Ala Asn
405 410 415

Lys Leu Val Pro Glu Gly Ile Glu Gly Arg Val Ala Tyr Lys Gly Ser
420 425 430

Val Ala Asp Met Ile Phe Gln Met Val Gly Gly Leu Arg Ser Gly Met
435 440 445

Gly Tyr Val Gly Ala Gly Asn Leu Thr Glu Leu His Glu Asn Ala Gln
450 455 460

Phe Ile Glu Met Ser Gly Ala Gly Leu Lys Glu Ser His Pro His Asp
465 470 475 480

Val Gln Ile Thr Asn Glu Ala Pro Asn Tyr Ser Val Gln
485 490

<210> 5
<211> 1206
<212> DNA
<213> Streptococcus suis

<400> 5
atgacaaaag aaaaatcaac cgtaccacga ttgcgcttcc ccggattcac ggacgcttgg 60

aaacagcgta agttggggga ggtggcggac ttttccataa aaacaaactc actttctaga 120

gataagttat catcgtattt ttatgaagtt cagaacattc attatgggga tattttaaca 180

aaatatgatg ctattttaga tgtatgtaac aaagaacttc catcaattat tggaagtacg 240

atttcagact ttgcagatgc tttacttagt gaaggagata ttgttttcgc ggatgcggca 300

gaagactcga ctgttgggaa agctattgaa gttcgaaatt ttaagggtaa gaatgttgtt 360

tctggtttac atacgatagt tgctaggccg aaagtttctt atgcccccta ctatttaggt 420

tatctaatta attcaactgc ataccataat caaattttac ctttaatgca ggggacaaaa 480

gtgagctcaa ttagtaaggc taatttaaaa tccacgacag tagtgttccc caccctccct 540

gaacaagaag ccatcggcag cttcttctcc gacctagatc agcttattac tcttcatcag 600

cgaaaattag atgatgttaa agaattgaag aaggctttgt tacagaaaat gtttccgaaa 660

gggaatggaa acgattttcc tgagctaaga ttcccagaat ttacggacgc ttggaaacag 720

cgtaagttgg gggagtttat gaaagaaagt aaaattttag gtagtaaagg agatatagca 780

aggaagttaa ctgtaagatt atgggggaga ggagttgttt ccaagaaaga aatttatagt 840

ggtagttcag ctacacagta ttatataaga aaatcaggcc agtttatata tgggaaatta 900

gacttttaa atcaagcttt tggaattatt ccaccagaac ttgatggtta tgaatcaact 960

cttgattcgc ctgcttttga tttattgaaa gggataaacg ggcaatttct gttagaattt 1020

gtatctcgca aagaatttta ttattatcaa ggaaatattg caaatggctc tagaaaagct 1080

aaaagaatcc acacagaaac ttttcttggc atgcctatct ccctccccac cctccctgaa 1140

caagaagcca tcggtagctt cttttccgac ctagatcagc ttattactct tcatcagcga 1200

aaatga 1206

<210> 6
<211> 401
<212> PRT
<213> Streptococcus suis

<400> 6

Met Thr Lys Glu Lys Ser Thr Val Pro Arg Leu Arg Phe Pro Gly Phe
1 5 10 15

Thr Asp Ala Trp Lys Gln Arg Lys Leu Gly Glu Val Ala Asp Phe Ser
20 25 30

Ile Lys Thr Asn Ser Leu Ser Arg Asp Lys Leu Ser Ser Tyr Phe Tyr
35 40 45

Glu Val Gln Asn Ile His Tyr Gly Asp Ile Leu Thr Lys Tyr Asp Ala
50 55 60

Ile Leu Asp Val Cys Asn Lys Glu Leu Pro Ser Ile Ile Gly Ser Thr
65 70 75 80

Ile Ser Asp Phe Ala Asp Ala Leu Leu Ser Glu Gly Asp Ile Val Phe
85 90 95

Ala Asp Ala Ala Glu Asp Ser Thr Val Gly Lys Ala Ile Glu Val Arg
100 105 110

Asn Phe Lys Gly Lys Asn Val Val Ser Gly Leu His Thr Ile Val Ala
115 120 125

Arg Pro Lys Val Ser Tyr Ala Pro Tyr Tyr Leu Gly Tyr Leu Ile Asn
130 135 140

Ser Thr Ala Tyr His Asn Gln Ile Leu Pro Leu Met Gln Gly Thr Lys

145 150 155 160

Val Ser Ser Ile Ser Lys Ala Asn Leu Lys Ser Thr Thr Val Val Phe
165 170 175

Pro Thr Leu Pro Glu Gln Glu Ala Ile Gly Ser Phe Phe Ser Asp Leu
180 185 190

Asp Gln Leu Ile Thr Leu His Gln Arg Lys Leu Asp Asp Val Lys Glu
195 200 205

Leu Lys Lys Ala Leu Leu Gln Lys Met Phe Pro Lys Gly Asn Gly Asn
210 215 220

Asp Phe Pro Glu Leu Arg Phe Pro Glu Phe Thr Asp Ala Trp Lys Gln
225 230 235 240

Arg Lys Leu Gly Glu Phe Met Lys Glu Ser Lys Ile Leu Gly Ser Lys
245 250 255

Gly Asp Ile Ala Arg Lys Leu Thr Val Arg Leu Trp Gly Arg Gly Val
260 265 270

Val Ser Lys Lys Glu Ile Tyr Ser Gly Ser Ser Ala Thr Gln Tyr Tyr
275 280 285

Ile Arg Lys Ser Gly Gln Phe Ile Tyr Gly Lys Leu Asp Phe Leu Asn
290 295 300

Gln Ala Phe Gly Ile Ile Pro Pro Glu Leu Asp Gly Tyr Glu Ser Thr
305 310 315 320

Leu Asp Ser Pro Ala Phe Asp Leu Leu Lys Gly Ile Asn Gly Gln Phe
325 330 335

Leu Leu Glu Phe Val Ser Arg Lys Glu Phe Tyr Tyr Tyr Gln Gly Asn
340 345 350

Ile Ala Asn Gly Ser Arg Lys Ala Lys Arg Ile His Thr Glu Thr Phe
355 360 365

Leu Gly Met Pro Ile Ser Leu Pro Thr Leu Pro Glu Gln Glu Ala Ile
370 375 380

Gly Ser Phe Phe Ser Asp Leu Asp Gln Leu Ile Thr Leu His Gln Arg
385 390 395 400

Lys

<210> 7
<211> 657
<212> DNA
<213> Streptococcus suis

<400> 7
atgcttggtg gttctctaca aatgattgca cttggttggg caaacattgg tgcagctgtt 60

gcacctgacg ctgctcttgc ttctgtagct gctgcaatca tcatgatcaa aggtggcgac 120

ttctcaactg aaggtattgc cgttgcaaca acaactgcta tccctcttgc cgtagctggt 180

cttttcctta caatgatcgt tcgtactatc tcagtcggtc ttgtacacgg tgctgacaat 240

gctgctaaag atgcaaactt cgctgctgta gaacgttacc acttgtttgc tcttctcctt 300

caaggtttgc gtatcgcagt tcctgcggct cttcttcttg caattcctgc agaagctgtt 360

caagcagttc ttgaatctat gccagcttgg ttgtcaggtg gtatggccgt tggtggtggt 420

atggttgttg ccgtaggtta tgctatggtt atcaacatga tggcaactcg tgaagtatgg 480

ccattcttcg cacttggttt tgcatttgct gctatcagca aattgacact tatcgcactt 540

ggtgttatcg gtgttgcaat cgctcttatc tacctcaact tgtcaaaaca aggcggatca 600

ggtaacggtg gtgcttcatc taacgcccca atcggcgaca tccttgaaga ctactaa 657

<210> 8
<211> 218
<212> PRT
<213> Streptococcus suis

<400> 8

Met Leu Gly Gly Ser Leu Gln Met Ile Ala Leu Gly Trp Ala Asn Ile
1 5 10 15

Gly Ala Ala Val Ala Pro Asp Ala Ala Leu Ala Ser Val Ala Ala Ala
20 25 30

Ile Ile Met Ile Lys Gly Gly Asp Phe Ser Thr Glu Gly Ile Ala Val
35 40 45

Ala Thr Thr Thr Ala Ile Pro Leu Ala Val Ala Gly Leu Phe Leu Thr
50 55 60

Met Ile Val Arg Thr Ile Ser Val Gly Leu Val His Gly Ala Asp Asn
65 70 75 80

Ala Ala Lys Asp Ala Asn Phe Ala Ala Val Glu Arg Tyr His Leu Phe
85 90 95

Ala Leu Leu Leu Gln Gly Leu Arg Ile Ala Val Pro Ala Ala Leu Leu
100 105 110

Leu Ala Ile Pro Ala Glu Ala Val Gln Ala Val Leu Glu Ser Met Pro
115 120 125

Ala Trp Leu Ser Gly Gly Met Ala Val Gly Gly Gly Met Val Val Ala
130 135 140

Val Gly Tyr Ala Met Val Ile Asn Met Met Ala Thr Arg Glu Val Trp
145 150 155 160

Pro Phe Phe Ala Leu Gly Phe Ala Phe Ala Ala Ile Ser Lys Leu Thr
165 170 175

Leu Ile Ala Leu Gly Val Ile Gly Val Ala Ile Ala Leu Ile Tyr Leu
180 185 190

Asn Leu Ser Lys Gln Gly Gly Ser Gly Asn Gly Gly Ala Ser Ser Asn
195 200 205

Ala Pro Ile Gly Asp Ile Leu Glu Asp Tyr
210 215

<210> 9
<211> 1293
<212> DNA
<213> Streptococcus suis

<400> 9
atgacatcag tagttgttgt aggaacccag tggggcgacg aaggtaaggg taaaattaca 60

gacttcttgt ctgctaatgc tgaagtaatc gcacgttatc aaggtggtga taacgctgga 120

cacactatcg ttatcgatgg cacgaagtat aaattgcact tgattccgtc aggaatcttc 180

ttcccagaaa agatttctgt aatcggtaat ggtgtggttg tcaatccaaa atctttggtg 240

aaggaaatca actatctcca tgattcaggt gtgacaacag ataatttgcg gatttcagac 300

cgcgcacatg tcatcttgcc ttatcacatc aaattggacc agttgcaaga agagtctaaa 360

ggtgagaata agattggtac aaccaacaag ggtatcggtc cagcctacat ggacaaggct 420

gcgcgtgttg gtatccgtat tgcagacctc ttggacaagg agatttttgc ggagcgtttg 480

agaacaaact tggctgagaa aaaccgcttg tttgagaaga tgtatgagtc aactccgatt 540

gagtttgacg aaatctttga agaatactat gcttacggtc aagaaatcaa aaagtatgta 600

acagacacat ctgttatttt gaacgatgcc ctcgaccaag gcaagcgtgt cttgtttgaa 660

ggtgcgcaag gagttatgtt ggatattgac caaggtacct atccattcgt tacttcttca 720

aacccagttg ctggtggtgt gacgatcggt agcggtgtcg gcccaagcaa gattgacaag 780

gttgttggtg tatgtaaggc ctacactagc cgtgttggtg acggaccatt tccgactgaa 840

ttgcacgatg aaatcggaga ccgtatccgc gaaatcggta aagagtacgg tacgacaact 900

ggccgtccac gccgtgtcgg ttggtttgac tcagtggtga tgcgccatag ccgccgtgtg 960

tcaggtatta ccaacttgtc cctcaactcg attgacgtct tgtcaggtct tgggaccttg 1020

aaaatctgcg tggcttatga cttggatggt gagcgtattg accactaccc agcaagtttg 1080

gagcaactca aacgttgcaa accaatctac gaagaaatgc caggttggtc tgaagacatc 1140

acaggtgtac gtagcctgga tgaattgcca gaagcggctc gcaactatgt tcgtcgtatc 1200

agcgaattgg taggcgttcg tatctcaacc ttctcagtag gacctggtcg tgaacagacc 1260

aacatccttg agagtgtatg gagcgcaaaa tag 1293

<210> 10
<211> 430
<212> PRT
<213> Streptococcus suis

<400> 10

Met Thr Ser Val Val Val Val Gly Thr Gln Trp Gly Asp Glu Gly Lys
1 5 10 15

Gly Lys Ile Thr Asp Phe Leu Ser Ala Asn Ala Glu Val Ile Ala Arg
20 25 30

Tyr Gln Gly Gly Asp Asn Ala Gly His Thr Ile Val Ile Asp Gly Thr
35 40 45

Lys Tyr Lys Leu His Leu Ile Pro Ser Gly Ile Phe Phe Pro Glu Lys
50 55 60

Ile Ser Val Ile Gly Asn Gly Val Val Val Asn Pro Lys Ser Leu Val
65 70 75 80

Lys Glu Ile Asn Tyr Leu His Asp Ser Gly Val Thr Thr Asp Asn Leu
85 90 95

Arg Ile Ser Asp Arg Ala His Val Ile Leu Pro Tyr His Ile Lys Leu
100 105 110

```
Asp Gln Leu Gln Glu Glu Ser Lys Gly Glu Asn Lys Ile Gly Thr Thr
        115                 120                 125

Asn Lys Gly Ile Gly Pro Ala Tyr Met Asp Lys Ala Ala Arg Val Gly
    130                 135                 140

Ile Arg Ile Ala Asp Leu Leu Asp Lys Glu Ile Phe Ala Glu Arg Leu
145                 150                 155                 160

Arg Thr Asn Leu Ala Glu Lys Asn Arg Leu Phe Glu Lys Met Tyr Glu
                165                 170                 175

Ser Thr Pro Ile Glu Phe Asp Glu Ile Phe Glu Glu Tyr Tyr Ala Tyr
            180                 185                 190

Gly Gln Glu Ile Lys Lys Tyr Val Thr Asp Thr Ser Val Ile Leu Asn
        195                 200                 205

Asp Ala Leu Asp Gln Gly Lys Arg Val Leu Phe Glu Gly Ala Gln Gly
    210                 215                 220

Val Met Leu Asp Ile Asp Gln Gly Thr Tyr Pro Phe Val Thr Ser Ser
225                 230                 235                 240

Asn Pro Val Ala Gly Gly Val Thr Ile Gly Ser Gly Val Gly Pro Ser
                245                 250                 255

Lys Ile Asp Lys Val Val Gly Val Cys Lys Ala Tyr Thr Ser Arg Val
            260                 265                 270

Gly Asp Gly Pro Phe Pro Thr Glu Leu His Asp Glu Ile Gly Asp Arg
        275                 280                 285

Ile Arg Glu Ile Gly Lys Glu Tyr Gly Thr Thr Thr Gly Arg Pro Arg
    290                 295                 300

Arg Val Gly Trp Phe Asp Ser Val Val Met Arg His Ser Arg Arg Val
305                 310                 315                 320

Ser Gly Ile Thr Asn Leu Ser Leu Asn Ser Ile Asp Val Leu Ser Gly
                325                 330                 335

Leu Gly Thr Leu Lys Ile Cys Val Ala Tyr Asp Leu Asp Gly Glu Arg
            340                 345                 350
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Ile Asp His Tyr Pro Ala Ser Leu Glu Gln Leu Lys Arg Cys Lys Pro
355 360 365

Ile Tyr Glu Glu Met Pro Gly Trp Ser Glu Asp Ile Thr Gly Val Arg
370 375 380

Ser Leu Asp Glu Leu Pro Glu Ala Ala Arg Asn Tyr Val Arg Arg Ile
385 390 395 400

Ser Glu Leu Val Gly Val Arg Ile Ser Thr Phe Ser Val Gly Pro Gly
405 410 415

Arg Glu Gln Thr Asn Ile Leu Glu Ser Val Trp Ser Ala Lys
420 425 430

<210> 11
<211> 1263
<212> DNA
<213> Streptococcus suis

<400> 11
atgaaacttt tggttgtcgg gtctggtggt cgtgaacatg ctatcgcaaa gaaattgtta 60

gagtctgagc aggtagagca ggtctttgtt gctcctggaa atgacggaat gactttagat 120

ggtatcgagt tagtcaatat cgggatctcc gaacattctg ctataatcaa ctttgctaag 180

gaaaatgacg ttgcttggac ttttgtgggt ccagacgatg ctctggcagc aggaatcgtt 240

gatgattttg aacaggcggg acttaaagct tttggcccta gtcgtctagc cgcggagcta 300

gagtggtcaa aagactttgc caaacaaatc atggtcaaat acggcattcc aacagcagcc 360

tttggcacat tttccaactt cgaagaagcc aaagcctaca tcgaagagca gggtgcacca 420

atcgtggtca aggcggacgg cttggcactg ggcaagggcg tggtcgtggc ggaaaccgtc 480

gagcaggcgg tcgaagcggc acgggagatg ctcttggaca acaagttcgg cgactcgggt 540

gcccgcgtgg tcatcgagga gttcttggcg ggtgaggagt tttccctctt tgctctggtc 600

aatggcgacc aattttacat tctgccgaca gcccaggacc acaagcgtgc ctttgacggc 660

gaccaaggtc ccaacacagg tggtatgggg gcttacgctc ctgttcccca cctgcctcaa 720

agcgtggtgg acacagcggt tgacaccatt gtcaagccga ttcttgaggg catgattgcg 780

gacggccggt cttatctggg cgtgctctat gctggcttga ttctgaccga ccaaggtccc 840

aaggtcatcg agttcaacgc ccgttttggc gacccagaaa cccagattat cctgcctcgc 900

ctgacctctg actttgctca gaacatcgac gacatcctcc acaaacgccc gacacagctg 960

acttggctgg atagtggcgt gacgctgggt gttgtcgtgg catcaaacgg ctaccctctg 1020

gactacgaaa aaggcgtaga gttgccagca aagaccgagg gcgacatcac gacctactat 1080

gcaggggctc gttttgcgga aaatagcaga gcactgcttt caaacggcgg tcgggtttat 1140

atgctagtca ccacagcaga caccgtccaa gacgcccaag aaaaaattta ctcggagctg 1200

aaaaatcaag ataccacagg cctcttttat cggacagata ttggaagtaa ggcagtaaaa 1260

taa 1263

<210> 12
<211> 420
<212> PRT
<213> Streptococcus suis

<400> 12

Met Lys Leu Leu Val Val Gly Ser Gly Gly Arg Glu His Ala Ile Ala
1 5 10 15

Lys Lys Leu Leu Glu Ser Glu Gln Val Glu Gln Val Phe Val Ala Pro
20 25 30

Gly Asn Asp Gly Met Thr Leu Asp Gly Ile Glu Leu Val Asn Ile Gly
35 40 45

Ile Ser Glu His Ser Ala Ile Ile Asn Phe Ala Lys Glu Asn Asp Val
50 55 60

Ala Trp Thr Phe Val Gly Pro Asp Asp Ala Leu Ala Ala Gly Ile Val
65 70 75 80

Asp Asp Phe Glu Gln Ala Gly Leu Lys Ala Phe Gly Pro Ser Arg Leu
85 90 95

Ala Ala Glu Leu Glu Trp Ser Lys Asp Phe Ala Lys Gln Ile Met Val
100 105 110

Lys Tyr Gly Ile Pro Thr Ala Ala Phe Gly Thr Phe Ser Asn Phe Glu
115 120 125

Glu Ala Lys Ala Tyr Ile Glu Glu Gln Gly Ala Pro Ile Val Val Lys
130 135 140

Ala Asp Gly Leu Ala Leu Gly Lys Gly Val Val Val Ala Glu Thr Val
145 150 155 160

Glu Gln Ala Val Glu Ala Ala Arg Glu Met Leu Leu Asp Asn Lys Phe
165 170 175

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Gly Asp Ser Gly Ala Arg Val Val Ile Glu Glu Phe Leu Ala Gly Glu
            180                 185                 190

Glu Phe Ser Leu Phe Ala Leu Val Asn Gly Asp Gln Phe Tyr Ile Leu
        195                 200                 205

Pro Thr Ala Gln Asp His Lys Arg Ala Phe Asp Gly Asp Gln Gly Pro
    210                 215                 220

Asn Thr Gly Gly Met Gly Ala Tyr Ala Pro Val Pro His Leu Pro Gln
225                 230                 235                 240

Ser Val Val Asp Thr Ala Val Asp Thr Ile Val Lys Pro Ile Leu Glu
                245                 250                 255

Gly Met Ile Ala Asp Gly Arg Ser Tyr Leu Gly Val Leu Tyr Ala Gly
            260                 265                 270

Leu Ile Leu Thr Asp Gln Gly Pro Lys Val Ile Glu Phe Asn Ala Arg
        275                 280                 285

Phe Gly Asp Pro Glu Thr Gln Ile Ile Leu Pro Arg Leu Thr Ser Asp
    290                 295                 300

Phe Ala Gln Asn Ile Asp Asp Ile Leu His Lys Arg Pro Thr Gln Leu
305                 310                 315                 320

Thr Trp Leu Asp Ser Gly Val Thr Leu Gly Val Val Val Ala Ser Asn
                325                 330                 335

Gly Tyr Pro Leu Asp Tyr Glu Lys Gly Val Glu Leu Pro Ala Lys Thr
            340                 345                 350

Glu Gly Asp Ile Thr Thr Tyr Tyr Ala Gly Ala Arg Phe Ala Glu Asn
        355                 360                 365

Ser Arg Ala Leu Leu Ser Asn Gly Gly Arg Val Tyr Met Leu Val Thr
    370                 375                 380

Thr Ala Asp Thr Val Gln Asp Ala Gln Glu Lys Ile Tyr Ser Glu Leu
385                 390                 395                 400

Lys Asn Gln Asp Thr Thr Gly Leu Phe Tyr Arg Thr Asp Ile Gly Ser
                405                 410                 415

Lys Ala Val Lys
```

420

<210> 13
<211> 1572
<212> DNA
<213> Streptococcus suis

<400> 13
atgtcaaacg tttatcacat tttgtttcaa ttttatcaaa aaacattgtt aaaacttgat 60

ttttcgcaag aaaacgttta ctatattaat gaaaaactag aaaatttgga gaaaattatg 120

gcatttacaa caggggagcg ttacagagct tatgctgact ggacacccga atacattgaa 180

aaaataaaga agaatacaga gagttctcct tggaggacaa atttccatat tgaaacacca 240

catggactgt taaatgatcc aaatggattt tcttatttta atggaaagtg gactcttttt 300

taccaatact tcccattcgg tgctgcgcac ggattgaaat cgtgggtcca tacagaatca 360

acagatttgg tccattttga agaaactggt acggttatgt acccagatac accacttgat 420

agccatggtg cctactctgg atctgccatg gaatttggcg acaagctctt cctcttctac 480

actggaaatg tccgcgatga aaattgggtt cgccatccat accaaatcgg tgccttgatg 540

gataaagatg gtaagattga aaagattgat aaagtcttga ttgagcaacc agaagatacc 600

accgaccatt tccgtgatcc acaaattttc aactataaag ggcaatatta cgctattgtc 660

ggtggacaaa atctggataa aaagggaatt gtcaaacttt ataaagctga aaataacgac 720

tacctcaact ggtcctatgt tggtgacctt gattttgcca atgatatgac cgcttatatg 780

atggaatgcc caaatattgc gttcgtcggt gagcaaccca tccttctcta ttgtccacaa 840

ggtttggaca aagaggtatt ggactatggc aatatctatc caaatatgta caagattggt 900

cagagtttcg ttcctgaaac agcaagcatt gtcgaaccga gcgaattgat caatctggac 960

tatggttttg agtgctatgc aacccaggcc tttaatgcac cagacggtcg aactctatct 1020

gttagctggt tggcacttcc agatgtggaa tacccaaccg atgcttatga ctaccaaggc 1080

tgtctgtcat tggttaaaga attaaccatc aaggacggta agctctacca atatccagtc 1140

gatgcgatta ccagtcttcg taaagaagct cagccatttt cagcccaaaa agaaaccaat 1200

aatgtttacg aattggaact tgatttttca gcgagcacga agcacgaaat cgtattgttc 1260

gcaaatgaag cagaaaaagg cttggtcttg tctgtagaca ctgaacaagg ccagattaca 1320

ttggaccgtg gcaactgtgg tcagccattc gcactcgatt tcggcacaag tcgctcttgc 1380

gagatttctc caggagctct tacagccaat atcttcatcg acaaatctgt ctttgaaatc 1440

tttatcaata aaggagagaa agtattttct ggtcgtgtct tccctgatgc ggatcagtct 1500

ggtatagtca ttacgactgg taatccgacc ggaacctact accatttaga ttatggtcgc 1560

aaaactaact ga 1572

<210> 14
<211> 523
<212> PRT
<213> Streptococcus suis

<400> 14

Met Ser Asn Val Tyr His Ile Leu Phe Gln Phe Tyr Gln Lys Thr Leu
1 5 10 15

Leu Lys Leu Asp Phe Ser Gln Glu Asn Val Tyr Tyr Ile Asn Glu Lys
20 25 30

Leu Glu Asn Leu Glu Lys Ile Met Ala Phe Thr Thr Gly Glu Arg Tyr
35 40 45

Arg Ala Tyr Ala Asp Trp Thr Pro Glu Tyr Ile Glu Lys Ile Lys Lys
50 55 60

Asn Thr Glu Ser Ser Pro Trp Arg Thr Asn Phe His Ile Glu Thr Pro
65 70 75 80

His Gly Leu Leu Asn Asp Pro Asn Gly Phe Ser Tyr Phe Asn Gly Lys
85 90 95

Trp Thr Leu Phe Tyr Gln Tyr Phe Pro Phe Gly Ala Ala His Gly Leu
100 105 110

Lys Ser Trp Val His Thr Glu Ser Thr Asp Leu Val His Phe Glu Glu
115 120 125

Thr Gly Thr Val Met Tyr Pro Asp Thr Pro Leu Asp Ser His Gly Ala
130 135 140

Tyr Ser Gly Ser Ala Met Glu Phe Gly Asp Lys Leu Phe Leu Phe Tyr
145 150 155 160

Thr Gly Asn Val Arg Asp Glu Asn Trp Val Arg His Pro Tyr Gln Ile
165 170 175

Gly Ala Leu Met Asp Lys Asp Gly Lys Ile Glu Lys Ile Asp Lys Val
180 185 190

Leu Ile Glu Gln Pro Glu Asp Thr Thr Asp His Phe Arg Asp Pro Gln
195 200 205

```
Ile Phe Asn Tyr Lys Gly Gln Tyr Tyr Ala Ile Val Gly Gly Gln Asn
    210                 215                 220

Leu Asp Lys Lys Gly Ile Val Lys Leu Tyr Lys Ala Glu Asn Asn Asp
225                 230                 235                 240

Tyr Leu Asn Trp Ser Tyr Val Gly Asp Leu Asp Phe Ala Asn Asp Met
                245                 250                 255

Thr Ala Tyr Met Met Glu Cys Pro Asn Ile Ala Phe Val Gly Glu Gln
            260                 265                 270

Pro Ile Leu Leu Tyr Cys Pro Gln Gly Leu Asp Lys Glu Val Leu Asp
        275                 280                 285

Tyr Gly Asn Ile Tyr Pro Asn Met Tyr Lys Ile Gly Gln Ser Phe Val
    290                 295                 300

Pro Glu Thr Ala Ser Ile Val Glu Pro Ser Glu Leu Ile Asn Leu Asp
305                 310                 315                 320

Tyr Gly Phe Glu Cys Tyr Ala Thr Gln Ala Phe Asn Ala Pro Asp Gly
                325                 330                 335

Arg Thr Leu Ser Val Ser Trp Leu Ala Leu Pro Asp Val Glu Tyr Pro
            340                 345                 350

Thr Asp Ala Tyr Asp Tyr Gln Gly Cys Leu Ser Leu Val Lys Glu Leu
        355                 360                 365

Thr Ile Lys Asp Gly Lys Leu Tyr Gln Tyr Pro Val Asp Ala Ile Thr
    370                 375                 380

Ser Leu Arg Lys Glu Ala Gln Pro Phe Ser Ala Gln Lys Glu Thr Asn
385                 390                 395                 400

Asn Val Tyr Glu Leu Glu Leu Asp Phe Ser Ala Ser Thr Lys His Glu
                405                 410                 415

Ile Val Leu Phe Ala Asn Glu Ala Glu Lys Gly Leu Val Leu Ser Val
            420                 425                 430

Asp Thr Glu Gln Gly Gln Ile Thr Leu Asp Arg Gly Asn Cys Gly Gln
        435                 440                 445
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Pro Phe Ala Leu Asp Phe Gly Thr Ser Arg Ser Cys Glu Ile Ser Pro
450 455 460

Gly Ala Leu Thr Ala Asn Ile Phe Ile Asp Lys Ser Val Phe Glu Ile
465 470 475 480

Phe Ile Asn Lys Gly Glu Lys Val Phe Ser Gly Arg Val Phe Pro Asp
485 490 495

Ala Asp Gln Ser Gly Ile Val Ile Thr Thr Gly Asn Pro Thr Gly Thr
500 505 510

Tyr Tyr His Leu Asp Tyr Gly Arg Lys Thr Asn
515 520

<210> 15
<211> 966
<212> DNA
<213> Streptococcus suis

<400> 15
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cgcgttatca ataaaaaagg atacctatct gaaaaaacca ttcgcaatgt cgaagaggct 120

atgcgtgaac taggctacaa acctaataat ttagctcgta gtttgcaagg taaatctgca 180

aaattagttg gtctcatctt cccaacaata aacaatattt tttattctga attgattagc 240

catttagaaa aagagctttt tgatcgaggc tataaaacca ttatttgcaa tagccagcac 300

gagtccgata aggaacgtga gtatctagaa atgttagcag ctaaccaagt agatgggatt 360

atctcgggca gtcacaatct cggtatccaa gactacgacc gtgtcgtagc tccaattatc 420

gcttttgacc gcaacctatc tccttccatt cccattgtct cttccgataa ttttgctggc 480

ggggtattag ctgcccaaac cttacaaaaa gcgggatgtc acaatccatt aatgattacg 540

gggaacgatg attccaattc acctaccggc cttcgccaag taggttttac atctattttg 600

aacaaagccg aagtttttca tatctccagt gatttctcac ctgtgcgtaa ggagatggaa 660

atccgagcca ttcttgaaaa gcaaaaacca aatggtatct ttgtatctgg cgatgcaacg 720

gctatgctgg tatggaacgt cattcgttca atgaaactgt ctattccaga agatattaaa 780

ttgattggtt atgatggaac cagtttcatc gaaaactact accctcaact gacaactatc 840

aagcaaccat taaccgaaat agctcgcctg ctggttgaac tattagttga taaaatcgaa 900

ggaaaaaaac tgacaaaaac agactatatc ctgccgatta gcctactagc aggagctagt 960

atataa 966

<210> 16
<211> 321
<212> PRT
<213> Streptococcus suis

<400> 16

Met Val Ala Lys Leu Thr Asp Val Ala Gln Leu Ala Gly Val Ser Pro
1 5 10 15

Thr Thr Val Ser Arg Val Ile Asn Lys Lys Gly Tyr Leu Ser Glu Lys
20 25 30

Thr Ile Arg Asn Val Glu Glu Ala Met Arg Glu Leu Gly Tyr Lys Pro
35 40 45

Asn Asn Leu Ala Arg Ser Leu Gln Gly Lys Ser Ala Lys Leu Val Gly
50 55 60

Leu Ile Phe Pro Thr Ile Asn Asn Ile Phe Tyr Ser Glu Leu Ile Ser
65 70 75 80

His Leu Glu Lys Glu Leu Phe Asp Arg Gly Tyr Lys Thr Ile Ile Cys
85 90 95

Asn Ser Gln His Glu Ser Asp Lys Glu Arg Glu Tyr Leu Glu Met Leu
100 105 110

Ala Ala Asn Gln Val Asp Gly Ile Ile Ser Gly Ser His Asn Leu Gly
115 120 125

Ile Gln Asp Tyr Asp Arg Val Val Ala Pro Ile Ile Ala Phe Asp Arg
130 135 140

Asn Leu Ser Pro Ser Ile Pro Ile Val Ser Ser Asp Asn Phe Ala Gly
145 150 155 160

Gly Val Leu Ala Ala Gln Thr Leu Gln Lys Ala Gly Cys His Asn Pro
165 170 175

Leu Met Ile Thr Gly Asn Asp Asp Ser Asn Ser Pro Thr Gly Leu Arg
180 185 190

Gln Val Gly Phe Thr Ser Ile Leu Asn Lys Ala Glu Val Phe His Ile
195 200 205

Ser Ser Asp Phe Ser Pro Val Arg Lys Glu Met Glu Ile Arg Ala Ile
210 215 220

Leu Glu Lys Gln Lys Pro Asn Gly Ile Phe Val Ser Gly Asp Ala Thr
225 230 235 240

Ala Met Leu Val Trp Asn Val Ile Arg Ser Met Lys Leu Ser Ile Pro
245 250 255

Glu Asp Ile Lys Leu Ile Gly Tyr Asp Gly Thr Ser Phe Ile Glu Asn
260 265 270

Tyr Tyr Pro Gln Leu Thr Thr Ile Lys Gln Pro Leu Thr Glu Ile Ala
275 280 285

Arg Leu Leu Val Glu Leu Leu Val Asp Lys Ile Glu Gly Lys Lys Leu
290 295 300

Thr Lys Thr Asp Tyr Ile Leu Pro Ile Ser Leu Leu Ala Gly Ala Ser
305 310 315 320

Ile

<210> 17
<211> 900
<212> DNA
<213> Streptococcus suis

<400> 17
atgaacgata aggaatttgg acagcgtgta cgtcaattac gagaatctgc tagtatgaca 60

cgtgaacagt tttgtgacga tgaactggaa ctctctgtgc gccaattaac tcgtattgaa 120

gcaggtgctt ccaagccgac tttttcaaag attcagtata ttgcaactcg tttaggtatg 180

ggactttacg agcttatgcc agattatgta tctttacccg aaagatattc caagctgaag 240

tttgatgtgc ttcgcacccc aacttatggt aatgaagatt tggcggaaaa gcgagatgcc 300

atgatgacag aaatctatga cgattattat gatgaattgc ctgaggagga gaagatagca 360

atagatgcga ttcaatcacg aattgatact ttagagtcag gtacagcagg ctttggaaaa 420

gagatactgg aagactactt tgaacaaatt tttcgcaaac gaaagtatga attgaatgat 480

ttgttgattg ttaggctcca tcttgaatat gttaggttat ctagctgtga ttcagaaata 540

tttagacagt ttttgaaaat tatagagcat ttacatgagc aaatcaatat catcaactca 600

aatgatttat ttgttttacg agacacgcta ttatcttgtg taaatatatt aggaagtaaa 660

aaatattacg aaccaatacc aaagatattt gacagtgtag ataagattat acagtcgaca 720

caagattttc agaaaaagcc cattgttagt gtattaaaat ggaaatatgc acttttttgtg 780

gataaggatc gggatgaggc agaaaagcat tatctagatg cggtgctatt tgcaaaattg 840

atagaaaata gagagttaga acagaagatt gaagaagatt ggagagttga caatcaataa 900

<210> 18
<211> 299
<212> PRT
<213> Streptococcus suis

<400> 18

Met Asn Asp Lys Glu Phe Gly Gln Arg Val Arg Gln Leu Arg Glu Ser
1 5 10 15

Ala Ser Met Thr Arg Glu Gln Phe Cys Asp Asp Glu Leu Glu Leu Ser
20 25 30

Val Arg Gln Leu Thr Arg Ile Glu Ala Gly Ala Ser Lys Pro Thr Phe
35 40 45

Ser Lys Ile Gln Tyr Ile Ala Thr Arg Leu Gly Met Gly Leu Tyr Glu
50 55 60

Leu Met Pro Asp Tyr Val Ser Leu Pro Glu Arg Tyr Ser Lys Leu Lys
65 70 75 80

Phe Asp Val Leu Arg Thr Pro Thr Tyr Gly Asn Glu Asp Leu Ala Glu
85 90 95

Lys Arg Asp Ala Met Met Thr Glu Ile Tyr Asp Asp Tyr Tyr Asp Glu
100 105 110

Leu Pro Glu Glu Glu Lys Ile Ala Ile Asp Ala Ile Gln Ser Arg Ile
115 120 125

Asp Thr Leu Glu Ser Gly Thr Ala Gly Phe Gly Lys Glu Ile Leu Glu
130 135 140

Asp Tyr Phe Glu Gln Ile Phe Arg Lys Arg Lys Tyr Glu Leu Asn Asp
145 150 155 160

Leu Leu Ile Val Arg Leu His Leu Glu Tyr Val Arg Leu Ser Ser Cys
165 170 175

Asp Ser Glu Ile Phe Arg Gln Phe Leu Lys Ile Ile Glu His Leu His
180 185 190

Glu Gln Ile Asn Ile Ile Asn Ser Asn Asp Leu Phe Val Leu Arg Asp
195 200 205

Thr Leu Leu Ser Cys Val Asn Ile Leu Gly Ser Lys Lys Tyr Tyr Glu
210 215 220

Pro Ile Pro Lys Ile Phe Asp Ser Val Asp Lys Ile Ile Gln Ser Thr
225 230 235 240

Gln Asp Phe Gln Lys Lys Pro Ile Val Ser Val Leu Lys Trp Lys Tyr
245 250 255

Ala Leu Phe Val Asp Lys Asp Arg Asp Glu Ala Glu Lys His Tyr Leu
260 265 270

Asp Ala Val Leu Phe Ala Lys Leu Ile Glu Asn Arg Glu Leu Glu Gln
275 280 285

Lys Ile Glu Glu Asp Trp Arg Val Asp Asn Gln
290 295

<210> 19
<211> 867
<212> DNA
<213> Streptococcus suis

<400> 19
atggttcgtt tgaaaaatat tttacttatt ttgcttggtg cagggctatt tgcctttggg 60

ctaaattatt taatcatgcc caatcgtcta ttcgagggtg gcgcaactgg attgacgctg 120

attatttact acctttttca tattcaacct tggataatga acattgtgat taatattcca 180

ttgttcatcc tcgggtggaa aatactcgga aagaagactc tctatctcag tatcttagga 240

acctttagcg ttacctttg gttggccatt tttgaaaaaa tccatttttc aatcaatcta 300

caacaagatt tgatactcgt tagtatcctg ggtgggattc ttatgggact aggattagga 360

accatcttcc gtgctggcgg aacgactggg ggaagcgata ttattgctcg tataggtcat 420

aaattcctac cttactccat tggacaaatc attcttgcag ttgacatcct catcctaact 480

ctgattgtta ttgtttttaa agacctccga acagttctct atactttaat gatggttgct 540

attgcttcca aggttattga ttttgtcacg gagggcggat atggtagcaa gggtgttatg 600

attgtttctc aaaaatctga ccaattggct caggctattg attgtgaaat cgagcgtggt 660

gtcaccttta tcaaagcaca aggtttctac agcaaagccg atgtcaacat gatttactcg 720

gtcatatata agagccaact ccaagagatg aaggagctta ttcatcgtat tgacccccac 780

gcatttatca ccattacaga cgcacatgaa gtactcggtg agggatttac tttagatcga 840

gataaaaaac caatagaacg ccattaa 867

<210> 20
<211> 288
<212> PRT
<213> Streptococcus suis

<400> 20

Met Val Arg Leu Lys Asn Ile Leu Leu Ile Leu Leu Gly Ala Gly Leu
1 5 10 15

Phe Ala Phe Gly Leu Asn Tyr Leu Ile Met Pro Asn Arg Leu Phe Glu
20 25 30

Gly Gly Ala Thr Gly Leu Thr Leu Ile Ile Tyr Tyr Leu Phe His Ile
35 40 45

Gln Pro Trp Ile Met Asn Ile Val Ile Asn Ile Pro Leu Phe Ile Leu
50 55 60

Gly Trp Lys Ile Leu Gly Lys Lys Thr Leu Tyr Leu Ser Ile Leu Gly
65 70 75 80

Thr Phe Ser Val Thr Phe Trp Leu Ala Ile Phe Glu Lys Ile His Phe
85 90 95

Ser Ile Asn Leu Gln Gln Asp Leu Ile Leu Val Ser Ile Leu Gly Gly
100 105 110

Ile Leu Met Gly Leu Gly Leu Gly Thr Ile Phe Arg Ala Gly Gly Thr
115 120 125

Thr Gly Gly Ser Asp Ile Ile Ala Arg Ile Gly His Lys Phe Leu Pro
130 135 140

Tyr Ser Ile Gly Gln Ile Ile Leu Ala Val Asp Ile Leu Ile Leu Thr
145 150 155 160

Leu Ile Val Ile Val Phe Lys Asp Leu Arg Thr Val Leu Tyr Thr Leu
165 170 175

Met Met Val Ala Ile Ala Ser Lys Val Ile Asp Phe Val Thr Glu Gly
180 185 190

Gly Tyr Gly Ser Lys Gly Val Met Ile Val Ser Gln Lys Ser Asp Gln
195 200 205

Leu Ala Gln Ala Ile Asp Cys Glu Ile Glu Arg Gly Val Thr Phe Ile
210 215 220

Lys Ala Gln Gly Phe Tyr Ser Lys Ala Asp Val Asn Met Ile Tyr Ser
225 230 235 240

Val Ile Tyr Lys Ser Gln Leu Gln Glu Met Lys Glu Leu Ile His Arg
245 250 255

Ile Asp Pro His Ala Phe Ile Thr Ile Thr Asp Ala His Glu Val Leu
260 265 270

Gly Glu Gly Phe Thr Leu Asp Arg Asp Lys Lys Pro Ile Glu Arg His
275 280 285

<210> 21
<211> 471
<212> DNA
<213> Streptococcus suis

<400> 21
atgcgaggaa ccccttact tttacaatta atttttgttt attacgtctt accaagtgtg 60

ggtattacat ttgaccgaat gcctgctgtg attctggcct ttacgctcaa ctatgcggcc 120

tatttctctg aaattttccg tggtggtatt gaagctattc caaaaggtca gtatgaagct 180

gctaaggtat tgaaatttac ccctgttcag accattcgct acattgtctt accacaggtt 240

gtgaaaattg ttctgccaag tgttttcaat gaagtcatga ctttggtaaa ggatacttct 300

ttggtctatg cacttggggt tagcgatttg ttgttggcta gtcgtactgc ggccaatcgt 360

gatgccagct tggcacctat gtttatcgca ggtgcaatct acctattgat gatcggtgcg 420

gtaacactga tttctaaaca agtagaaaag aaatttgatt attatagata g 471

<210> 22
<211> 156
<212> PRT
<213> Streptococcus suis

<400> 22

Met Arg Gly Thr Pro Leu Leu Leu Gln Leu Ile Phe Val Tyr Tyr Val
1 5 10 15

Leu Pro Ser Val Gly Ile Thr Phe Asp Arg Met Pro Ala Val Ile Leu
20 25 30

Ala Phe Thr Leu Asn Tyr Ala Ala Tyr Phe Ser Glu Ile Phe Arg Gly

35 40 45

Gly Ile Glu Ala Ile Pro Lys Gly Gln Tyr Glu Ala Ala Lys Val Leu
50 55 60

Lys Phe Thr Pro Val Gln Thr Ile Arg Tyr Ile Val Leu Pro Gln Val
65 70 75 80

Val Lys Ile Val Leu Pro Ser Val Phe Asn Glu Val Met Thr Leu Val
85 90 95

Lys Asp Thr Ser Leu Val Tyr Ala Leu Gly Val Ser Asp Leu Leu Leu
100 105 110

Ala Ser Arg Thr Ala Ala Asn Arg Asp Ala Ser Leu Ala Pro Met Phe
115 120 125

Ile Ala Gly Ala Ile Tyr Leu Leu Met Ile Gly Ala Val Thr Leu Ile
130 135 140

Ser Lys Gln Val Glu Lys Lys Phe Asp Tyr Tyr Arg
145 150 155

<210> 23
<211> 708
<212> DNA
<213> Streptococcus suis

<400> 23
atgagtaagt ataaaaaagt ttatcaagat attaagaaaa aaatcgaaga gcaggtttgg 60

tcaactggac aggccctacc gacagaaaat gaattaatgg aagtgtattc ctactccaag 120

gacaccattc gcaaagcact atctcttctt gaaatggatg gttatattca aaagaaacag 180

gggaaatctt ctattgttat cgaacatggt ctaatgaagg accaatatct ttctgaaatt 240

aaaacagcgg gcgaattgaa caaaagatca cagcaccaaa ttcagaccca gcttacttcg 300

ctttatatta ttcaaggtca agaagattta atgtcaactt tcgaagttga tgataaggta 360

gatttctatc gagtgagtcg ggtacggaca attgacggtg agcggttgga atatgacatt 420

tcctattttg accgtagggt cgtcccttat ctcagcaaag aaattgccga aagctctatt 480

taccgctact tagaagaaga actccattta acaatttctc attccagacg tgaaatttcc 540

ttccgttttg caaacgaaga agaaaaacaa ttgatggact taggagacta tgacatggtc 600

gtcgtggtca ctagcatcac ctatctttca aatggtcaag ctttccagta tggaacaatt 660

agttaccgtc cagacaaagt tagctttgta tcaatggcga aacggtaa 708

<210> 24
<211> 235
<212> PRT
<213> Streptococcus suis

<400> 24

Met Ser Lys Tyr Lys Lys Val Tyr Gln Asp Ile Lys Lys Lys Ile Glu
1 5 10 15

Glu Gln Val Trp Ser Thr Gly Gln Ala Leu Pro Thr Glu Asn Glu Leu
20 25 30

Met Glu Val Tyr Ser Tyr Ser Lys Asp Thr Ile Arg Lys Ala Leu Ser
35 40 45

Leu Leu Glu Met Asp Gly Tyr Ile Gln Lys Lys Gln Gly Lys Ser Ser
50 55 60

Ile Val Ile Glu His Gly Leu Met Lys Asp Gln Tyr Leu Ser Glu Ile
65 70 75 80

Lys Thr Ala Gly Glu Leu Asn Lys Arg Ser Gln His Gln Ile Gln Thr
85 90 95

Gln Leu Thr Ser Leu Tyr Ile Ile Gln Gly Gln Glu Asp Leu Met Ser
100 105 110

Thr Phe Glu Val Asp Asp Lys Val Asp Phe Tyr Arg Val Ser Arg Val
115 120 125

Arg Thr Ile Asp Gly Glu Arg Leu Glu Tyr Asp Ile Ser Tyr Phe Asp
130 135 140

Arg Arg Val Val Pro Tyr Leu Ser Lys Glu Ile Ala Glu Ser Ser Ile
145 150 155 160

Tyr Arg Tyr Leu Glu Glu Glu Leu His Leu Thr Ile Ser His Ser Arg
165 170 175

Arg Glu Ile Ser Phe Arg Phe Ala Asn Glu Glu Glu Lys Gln Leu Met
180 185 190

Asp Leu Gly Asp Tyr Asp Met Val Val Val Val Thr Ser Ile Thr Tyr
195 200 205

Leu Ser Asn Gly Gln Ala Phe Gln Tyr Gly Thr Ile Ser Tyr Arg Pro

210 215 220

Asp Lys Val Ser Phe Val Ser Met Ala Lys Arg
225 230 235

<210> 25
<211> 27
<212> DNA
<213> Synthetic

<400> 25
catgaattcc atggtaccca ttctaac 27

<210> 26
<211> 27
<212> DNA
<213> Synthetic

<400> 26
ctagaattcc taggtaccta caacctc 27

<210> 27
<211> 27
<212> DNA
<213> Synthetic

<400> 27
catgaattcc atggtaccca ttctaac 27

<210> 28
<211> 35
<212> DNA
<213> Synthetic

<220>
<221> misc_feature
<222> (21)..(30)
<223> n is a, c, g, or t

<400> 28
ggccacgcgt cgactagtac nnnnnnnnnn catat 35

<210> 29
<211> 20
<212> DNA
<213> Synthetic

<400> 29
ggccacgcgt cgactagtac 20

<210> 30
<211> 35
<212> DNA

<213> Synthetic

<220>
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<222> (21)..(30)
<223> n is a, c, g, or t

<400> 30
ggccacgcgt cgactagtac nnnnnnnnnn acgcc 35

<210> 31
<211> 36
<212> DNA
<213> Synthetic

<220>
<221> misc_feature
<222> (21)..(30)
<223> n is a, c, g, or t

<400> 31
ggccacgcgt cgactagtac nnnnnnnnnn atgcat 36

<210> 32
<211> 36
<212> DNA
<213> Synthetic

<220>
<221> misc_feature
<222> (21)..(30)
<223> n is a, c, g, or t

<400> 32
ggccacgcgt cgactagtac nnnnnnnnnn gttaac 36

<210> 33
<211> 36
<212> DNA
<213> Synthetic

<220>
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<222> (21)..(30)
<223> n is a, c, g, or t

<400> 33
ggccacgcgt cgactagtac nnnnnnnnnn ttcgaa 36

<210> 34
<211> 36
<212> DNA
<213> Synthetic

<220>
<221> misc_feature
<222> (21)..(30)
<223> n is a, c, g, or t

<400> 34
ggccacgcgt cgactagtac nnnnnnnnnn gatatc 36

<210> 35
<211> 36
<212> DNA
<213> Synthetic

<220>
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<222> (21)..(30)
<223> n is a, c, g, or t

<400> 35
ggccacgcgt cgactagtac nnnnnnnnnn tgatca 36

<210> 36
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<220>
<221> misc_feature
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<223> n is a, c, g, or t

<400> 36
ggccacgcgt cgactagtac nnnnnnnnnn cagctg 36

<210> 37
<211> 25
<212> DNA
<213> Synthetic

<400> 37
aggagttctt gaatttcacg atagc 25

<210> 38
<211> 25
<212> DNA
<213> Synthetic

<400> 38
cgattaaggg catggaaaca attcg 25

<210> 39
<211> 23

<212> DNA
<213> Synthetic

<400> 39
aaagcgccct ctattggttc tgc 23

<210> 40
<211> 23
<212> DNA
<213> Synthetic

<400> 40
ggtttgatgt tgcgattaat agc 23

<210> 41
<211> 23
<212> DNA
<213> Synthetic

<400> 41
gaaggagaaa actcaccgag gca 23

<210> 42
<211> 23
<212> DNA
<213> Synthetic

<400> 42
agcacatcat catcataagg ttt 23

<210> 43
<211> 23
<212> DNA
<213> Synthetic

<400> 43
tcattcacta ccaatttggg atg 23

<210> 44
<211> 20
<212> DNA
<213> Synthetic

<400> 44
ccgaagaaat ggaacgctct 20

<210> 45
<211> 22
<212> DNA
<213> Synthetic

<400> 45
cagcgaggat atatcaatga ac 22

<210> 46
<211> 20
<212> DNA
<213> Synthetic

<400> 46
tggtgagagt gaccgctagg 20

<210> 47
<211> 20
<212> DNA
<213> Synthetic

<400> 47
ggatgtagcg gtctttctgg 20

<210> 48
<211> 20
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<213> Synthetic

<400> 48
ggcgatgtat ggctaggttt 20

<210> 49
<211> 20
<212> DNA
<213> Synthetic

<400> 49
gtggggctct gtgtttggtg 20

<210> 50
<211> 20
<212> DNA
<213> Synthetic

<400> 50
ggtggggtgt cggggtgggg 20

<210> 51
<211> 20
<212> DNA
<213> Synthetic

<400> 51
gttgcggtat cgctatttgt 20

<210> 52
<211> 20
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<213> Synthetic

<400> 52
tggcggggtt gcgagttcta 20

<210> 53
<211> 20
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<400> 53
ggtttagcga ttttgggggga 20

<210> 54
<211> 20
<212> DNA
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<400> 54
ggagctgggt gtatagatgg 20

<210> 55
<211> 20
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<400> 55
agggggtgtg gtgtggggat 20

<210> 56
<211> 20
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<400> 56
agtgatgggt cggggttgtg 20

<210> 57
<211> 20
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<213> Synthetic

<400> 57
ggcgctagta ggtttggtgt 20

<210> 58
<211> 20
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<400> 58
tgtgcgatgg gttgttgggg 20

<210> 59
<211> 20
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<213> Synthetic

<400> 59
atggggcggt cgggatatgt 20

<210> 60
<211> 20
<212> DNA
<213> Synthetic

<400> 60
tggggttata gtgattttgg 20

<210> 61
<211> 20
<212> DNA
<213> Synthetic

<400> 61
gtatatggag agctgtgtag 20

<210> 62
<211> 20
<212> DNA
<213> Synthetic

<400> 62
gtggcatgtt tgtgtgaggg 20

<210> 63
<211> 20
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<213> Synthetic

<400> 63
ggggtgcggt ttggttattg 20

<210> 64
<211> 20
<212> DNA
<213> Synthetic

<400> 64
aggtgcgagc tcggtctgta 20

<210> 65
<211> 20
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<213> Synthetic

<400> 65
atctcggggt cgggggatgg 20

<210> 66
<211> 20
<212> DNA

<213> Synthetic

<400> 66
gcgtggtgtg cggtctattt 20

<210> 67
<211> 20
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<400> 67
tgtggttggt gtgtagagtg 20

<210> 68
<211> 20
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<213> Synthetic

<400> 68
cgtgtgtggt gtttgggggt 20

<210> 69
<211> 20
<212> DNA
<213> Synthetic

<400> 69
gtgggtgatg tgtggatggc 20

<210> 70
<211> 20
<212> DNA
<213> Synthetic

<400> 70
agtgggttgt tgtgtcgaga 20

<210> 71
<211> 20
<212> DNA
<213> Synthetic

<400> 71
ggtcgcgggt gtttctgggg 20

<210> 72
<211> 20
<212> DNA
<213> Synthetic

<400> 72
ggcggggttg ttaggggtcg 20

<210> 73

<211> 20
<212> DNA
<213> Synthetic

<400> 73
ggtgggcgcg ggggctgggg 20

<210> 74
<211> 20
<212> DNA
<213> Synthetic

<400> 74
ggagggtttg tttgggcgtt 20

<210> 75
<211> 20
<212> DNA
<213> Synthetic

<400> 75
atcgggggag gggttgcgct 20

<210> 76
<211> 20
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<213> Synthetic

<400> 76
cggggtgtgg ggatagtggt 20

<210> 77
<211> 20
<212> DNA
<213> Synthetic

<400> 77
ctttggaggg atgttttgag 20

<210> 78
<211> 20
<212> DNA
<213> Synthetic

<400> 78
ctctttgcgc tttttagtgg 20

<210> 79
<211> 20
<212> DNA
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<400> 79
tggagtggta tggctcgcgg 20

<210> 80
<211> 20
<212> DNA
<213> Synthetic

<400> 80
tagttgctgg tggtttgtgt 20

<210> 81
<211> 20
<212> DNA
<213> Synthetic

<400> 81
gtgcgctcgc gatcgatgtc 20

<210> 82
<211> 20
<212> DNA
<213> Synthetic

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acttgggttg ctttagcagc tgaaagcttg aaagatagtg atgtaaaagt ttgtacggtt 180

attggtttcc ctctcggagc aaacacacct gctgtaaaag cgttcgaaac aaaagatgca 240

atttcaaatg gagcagatga gattgacgtg gttatcaata tcggtgctct gaaaactgga 300

aattacgatt tggttttgga ggatattaaa gctgttgtag cagcaagtgg tgataaacta 360

gtcaaagtta tcatcgaagc atgcctcctt actgatgatg agaaagtgaa agcttgtcaa 420

ttatctcaag aagctggtgc ggactatgtt aaaacatcta caggattttc tacaggcggt 480

gctacagtag cggatgttgc cttaatgaga aaaacagtgg gtcctgacat gggagtgaag 540

gcttcaggtg gtgcacgttc gtatgaagat gccattgcct ttatcgaagc aggagcgtct 600

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<213> Streptococcus suis

<400> 143

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Phe Ala Ser Val Cys Val Asn Pro Thr Trp Val Ala Leu Ala Ala Glu
35 40 45

Ser Leu Lys Asp Ser Asp Val Lys Val Cys Thr Val Ile Gly Phe Pro

50 55 60

Leu Gly Ala Asn Thr Pro Ala Val Lys Ala Phe Glu Thr Lys Asp Ala
65 70 75 80

Ile Ser Asn Gly Ala Asp Glu Ile Asp Val Val Ile Asn Ile Gly Ala
85 90 95

Leu Lys Thr Gly Asn Tyr Asp Leu Val Leu Glu Asp Ile Lys Ala Val
100 105 110

Val Ala Ala Ser Gly Asp Lys Leu Val Lys Val Ile Ile Glu Ala Cys
115 120 125

Leu Leu Thr Asp Asp Glu Lys Val Lys Ala Cys Gln Leu Ser Gln Glu
130 135 140

Ala Gly Ala Asp Tyr Val Lys Thr Ser Thr Gly Phe Ser Thr Gly Gly
145 150 155 160

Ala Thr Val Ala Asp Val Ala Leu Met Arg Lys Thr Val Gly Pro Asp
165 170 175

Met Gly Val Lys Ala Ser Gly Gly Ala Arg Ser Tyr Glu Asp Ala Ile
180 185 190

Ala Phe Ile Glu Ala Gly Ala Ser Arg Ile Gly Ala Ser Ser Gly Val
195 200 205

Ala Ile Met Asn Gly Ala Gln Ala Asp Gly Asp Tyr
210 215 220

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gaaaatgtgg acagcttgtc agagccagct gttgaactct cttccgtagt gaacgaacat 180

gtggaaaaga ccgaagctga agaaggaatg tcaccaattt cagcagacga gaaagcggtg 240

accgaagagt tgaatggaga agcatctgta tcagatagta gagaagaaaa actgccttca 300

gctccgtctg ataatccaga aaataaacta gaggatacag aggaagcgga agataaagag 360

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attcgtccga aagagattaa gtttgacaac tgggatcagg tgcttgcttg ggagccgggt 480

gctcgtccag atgatgattt gaaccgcgct tctgtagagt tggttgagcg ctttagaggg 540

cacgtcgtca atgagagagc caatgagaat gcaaaggtag aagctctttc aaataccaac 600

tctaaggcga aagaccatgc ctctgttggg ggagaagagt ttaaagccta tgcctttgat 660

tactggcagt atattgattc aatggtattt tgggaaggct tgattcctag cccagacgtc 720

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ataaatcaag agactacagg aggcttggtt caaccactag gggaaacaat gagaaacttc 960

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<212> PRT
<213> Streptococcus suis

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Phe Thr Ile Gly Ala Cys Ser Val Leu Ile Gly Ala Thr Leu Phe Gly
20 25 30

Thr Gln Val Ser Ala Asp Gln Leu Glu Asn Val Asp Ser Leu Ser Glu
35 40 45

Pro Ala Val Glu Leu Ser Ser Val Val Asn Glu His Val Glu Lys Thr
50 55 60

Glu Ala Glu Glu Gly Met Ser Pro Ile Ser Ala Asp Glu Lys Ala Val
65 70 75 80

Thr Glu Glu Leu Asn Gly Glu Ala Ser Val Ser Asp Ser Arg Glu Glu
85 90 95

Lys Leu Pro Ser Ala Pro Ser Asp Asn Pro Glu Asn Lys Leu Glu Asp
100 105 110

Thr Glu Glu Ala Glu Asp Lys Glu Val Asp Ser Ser Asp Ser Gly Leu
115 120 125

Leu Leu Ser Asp Phe Pro Gln Val Glu Asp Gln Gly Ile Arg Pro Lys
130 135 140

Glu Ile Lys Phe Asp Asn Trp Asp Gln Val Leu Ala Trp Glu Pro Gly

145 150 155 160

Ala Arg Pro Asp Asp Asp Leu Asn Arg Ala Ser Val Glu Leu Val Glu
165 170 175

Arg Phe Arg Gly His Val Val Asn Glu Arg Ala Asn Glu Asn Ala Lys
180 185 190

Val Glu Ala Leu Ser Asn Thr Asn Ser Lys Ala Lys Asp His Ala Ser
195 200 205

Val Gly Gly Glu Glu Phe Lys Ala Tyr Ala Phe Asp Tyr Trp Gln Tyr
210 215 220

Ile Asp Ser Met Val Phe Trp Glu Gly Leu Ile Pro Ser Pro Asp Val
225 230 235 240

Ile Asp Ala Gly His Arg Asn Gly Val Pro Val Leu Gly Thr Ile Phe
245 250 255

Tyr Asn Trp Ser Ser Ser Ile Glu Asp Gln Glu Lys Phe Val Ser Ser
260 265 270

Met Arg Gln Asp Pro Asp Gly Ser Phe Pro Val Ala Arg Lys Leu Val
275 280 285

Asp Met Ala Lys Tyr Tyr Gly Phe Asp Gly Tyr Phe Ile Asn Gln Glu
290 295 300

Thr Thr Gly Gly Leu Val Gln Pro Leu Gly Glu Thr Met Arg Asn Phe
305 310 315 320

Met Leu Tyr Thr Lys Glu Tyr Ala Glu Ser Ile Gly Tyr Pro Ile Thr
325 330 335

Tyr Ser Trp Tyr Asp Ala Met Thr Tyr Glu Tyr Gly Arg Tyr His Glu
340 345 350

Asp Gly Leu Gly Glu Tyr Asn Tyr Pro Phe Met Glu Lys Glu Gly Asp
355 360 365

Lys Val Pro Ala Asp His Phe Phe Ala Asn Phe Asn Trp Thr Arg Ala
370 375 380

Lys Asn Asn Tyr Ser Ile Glu Val Ala Lys Arg Ile Gly Arg Asn Pro
385 390 395 400

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Phe Asp Ile Phe Ala Gly Phe Glu Leu Gln Arg Gly Gly Ser Tyr Lys
                405                 410                 415

Thr Gln Ile Asn Trp Asp Ala Leu Phe Asp Glu Asn Gly Lys Leu Lys
            420                 425                 430

Leu Ser Leu Gly Leu Phe Ala Pro Asp Thr Ile Thr Ser Leu Gly Ala
        435                 440                 445

Thr Gly Glu Asp Tyr His Glu Asn Glu Asn Ile Phe Phe Thr Gly Tyr
    450                 455                 460

Gln Gly Asp Pro Thr Lys Gln Lys Pro Ser Asp Lys Asn Trp Tyr Gly
465                 470                 475                 480

Leu Ala Asn Leu Ile Ala Asp Lys Thr Ala Ile Thr Ser Ser Asn Phe
                485                 490                 495

Asn Ser Ser Phe Asn Thr Gly His Gly Lys Lys Trp Phe Val Asp Gly
            500                 505                 510

Lys Val Ala Lys Asp Gly Glu Trp Asn Tyr Arg Ser Val Ser Gly Ile
        515                 520                 525

Leu Pro Thr Trp Arg Trp Trp Ile Glu Ala Ser Gly Glu Lys Leu Ala
    530                 535                 540

Ala Ala Tyr Asp Phe Asp Asp Ala Tyr Asn Gly Gly Asn Ser Leu Arg
545                 550                 555                 560

Phe Thr Gly Asp Leu Glu Lys Asp Gly Lys Gln Glu Ile Lys Leu Tyr
                565                 570                 575

Ser Thr Lys Ile Pro Leu Ser Glu Thr Ser Arg Leu Arg Val Ala His
            580                 585                 590

Lys Gly Gly Gln Gly Ala Asn Ile Ser Leu Ala Leu Ala Leu Glu Pro
        595                 600                 605

Asp Tyr Gln Phe Ala Asn Lys Glu Ala Trp Arg Leu Leu Lys Leu Ser
    610                 615                 620

Gly Asp Trp Gln Asp Gln Thr Phe Asp Leu Ser Ser Leu Ala Gly Lys
625                 630                 635                 640
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Thr Val Tyr Gly Ile Gln Val Val Val Glu Asn Glu Ala Ala Leu Ser
                645                 650                 655

Asp Phe Asp Phe Arg Leu Gly Gln Leu Ala Ile Tyr Asp Gln Glu Asn
            660                 665                 670

Ser Pro Thr Ala Pro Lys Asp Gly Gln Val Leu Ala Lys Arg Leu Lys
        675                 680                 685

Asn Ala Gln Asp Ala Glu Ala Val Ile Ser Phe Thr Gly Thr Asp Asp
    690                 695                 700

Ala Asp Tyr Tyr Glu Val Tyr Ala Gln Val Asp Gly Gln Trp Lys Leu
705                 710                 715                 720

Leu Thr Gly Ser Ser Asn Thr Arg Ile Tyr Leu Pro Gln Leu Val Arg
                725                 730                 735

Ser Ala Gln Ala Glu Gly Arg Thr Gln Ala Leu Lys Val His Ala Val
            740                 745                 750

Gly Lys Asn Gly Leu Arg Ser Glu Ala Gly Glu Phe Ile Phe Asp Trp
        755                 760                 765

Glu Met Thr Val Lys Asp Thr Ser Leu Pro Lys Pro Ser Ala Glu Asn
    770                 775                 780

Ile Val Leu Gly Ala Glu Val Ile Gly Ser Ser Phe Ala Lys Lys Glu
785                 790                 795                 800

Gly Gly Glu Gly Ile Glu Gly Met Leu Asn Gly Thr Ile Thr Ser Leu
                805                 810                 815

Ser Asp Lys Trp Ser Ser His Gln Leu Ser Gly His Val Asp Ile Arg
            820                 825                 830

Leu Thr Gln Pro Arg Thr Val Val Arg Trp Ala Met Asp His Ala Gly
        835                 840                 845

Ala Gly Gly Glu Ser Val Asn Asp Gly Leu Met Asn Thr Lys Asp Phe
    850                 855                 860

Asp Leu Tyr Tyr Lys Asn Glu Asn Gly Asp Trp Val Leu Ala Lys Glu
865                 870                 875                 880
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Val Arg Gly Asn Arg Asp His Val Thr Asp Ile Val Leu Asp Lys Pro
                885                 890                 895

Ile Arg Ala Gln Glu Trp Arg Leu Asp Val Leu Thr Ser Asp Asn Gly
            900                 905                 910

Thr Pro Trp Lys Ala Ile Arg Ile Tyr Asn Trp Arg Met Tyr Glu Glu
        915                 920                 925

Leu Asp Met Glu Thr Pro Asn Ile Pro Met Thr His Ala Val Ala Arg
    930                 935                 940

His Leu Gly Asn His Gln Ile Gln Val Gly Phe Lys Asp Val Pro Ala
945                 950                 955                 960

Asn Arg Lys Ile Ala Leu Tyr Ser Ser Pro Asp Ala Val Lys Pro Leu
                965                 970                 975

Ala Glu Leu Glu Thr Thr Glu Ser Gly Asn Leu Ile Phe Asp Pro Ile
            980                 985                 990

Arg Phe Glu Ser Leu Pro Glu Phe  Ile Tyr Tyr Arg Thr  Leu Glu Glu
        995                 1000                1005

Gly Lys  Asp Trp Ser Asn Leu  Leu Ala Ile Arg Val  Pro Gln Gly
    1010                1015                1020

Glu Lys  Val Val Lys Ala Met  Glu Trp Ile Ala Pro  Leu Glu Lys
    1025                1030                1035

Lys Val  Tyr Arg Gln Gly Ser  Pro Leu Ser Leu Ala  Gly Ala Gln
    1040                1045                1050

Phe Arg  Val Val Phe Glu Gly  Asp Trp Pro Ala Glu  Arg Val Asn
    1055                1060                1065

Thr Thr  Asn Pro Ala Val Thr  Val Arg Gly Phe Asp  Pro Gln Lys
    1070                1075                1080

Leu Gly  Glu Gln Thr Leu Thr  Val Ser Tyr Leu Gly  Glu Thr Leu
    1085                1090                1095

Ala Gln  Pro Leu Met Val Tyr  Val Val Glu Asp Ile  Ala Gln Gly
    1100                1105                1110

Glu Lys  Lys Ala Val Gly Leu  Glu Ile Gln Gln Leu  Pro Lys Val
```

1115 1120 1125

Gln Tyr Val Val Gly Asp Asp Leu Asp Val Thr Asn Gly Arg Phe
1130 1135 1140

Ala Ile Ile Tyr Asp Asp Glu Ser Thr Gln Ser Phe Ser Leu Thr
1145 1150 1155

Glu Ala Gly Val Glu Ile Val Gly Phe Asp Ser Arg Lys Glu Gly
1160 1165 1170

Arg Gln Ser Leu Gly Leu Arg Tyr Ala Gly Leu Glu Thr Ser Phe
1175 1180 1185

Asp Val Leu Val Ser Pro Lys Pro Ile Val Asn Asp Glu Tyr Leu
1190 1195 1200

Lys Gln Lys Ile Ala Glu Ile Glu Ala Leu Gln Thr Gln Thr Ala
1205 1210 1215

Tyr Leu Tyr Ser Ser Glu Gln Thr Gln Leu Ala Leu Gln Thr Ala
1220 1225 1230

Leu Glu Glu Ala Lys Ser Val Val Ala Asp Thr Asn Arg Thr Val
1235 1240 1245

Glu Gln Val Glu Ala Ala Gln Thr Leu Leu Glu Lys Gln Leu Gly
1250 1255 1260

Glu Leu Asp Gly Glu Lys Leu Tyr Gln Ala Asp Leu Gly Arg Leu
1265 1270 1275

Asn Gln Leu Ile Gln Glu Val Lys Gly Ile Asp Arg Pro Ile Asn
1280 1285 1290

Ser Ser Glu Leu Ser Ser Leu Leu Glu Lys Thr Glu Lys Ile Leu
1295 1300 1305

Gly Ser Asp Ser Ile Arg Pro Asp Glu Met Lys Glu Leu Leu Ser
1310 1315 1320

Glu Trp Thr Asp Leu Phe Glu Gln Ser Ser Leu Val Thr His Val
1325 1330 1335

Gly Thr Arg Asp Ser Met Glu Ser Lys Asn Leu Leu Ala Pro Glu
1340 1345 1350

Lys Pro Arg Met Asp Leu Thr Tyr Glu Leu Val Pro Arg Gln Ile
1355 1360 1365

Ile Thr Gln Thr Ser Thr Asp Leu Pro Leu Gly Lys Glu Arg Ile
1370 1375 1380

Val Gln Glu Gly Ala Asp Gly Gln Met Met Ile Ala His Leu Val
1385 1390 1395

Tyr Ser Asp Gly Arg Arg Glu Leu Tyr Ser Arg Ile Val Ala Asp
1400 1405 1410

Glu Ser Gln Pro Lys Ile Val Glu Val Gly Ser Gly Leu Pro Ile
1415 1420 1425

Gln Gln Glu Lys Thr Leu Met Val Glu Asn Ser Leu Gln Val Glu
1430 1435 1440

Lys Gln Ile Arg Asp Ala Ser Leu Gln Lys Ser Gly Leu Pro Gln
1445 1450 1455

Thr Gly Asp Thr Tyr Gln Glu Lys Tyr Val Phe Leu Gly Leu Ile
1460 1465 1470

Gly Val Ala Leu Ala Gly Leu Ser Gln Leu Ala Lys Leu Arg Lys
1475 1480 1485

Gln Gly Glu
1490

<210> 146
<211> 1017
<212> DNA
<213> Streptococcus suis

<400> 146
atggtttata ttattgcaga aattggttgt aatcacaacg gtgatgttca tctagcacgg 60

aaaatggtag aagttgccgt tgattgtggt gtggatgccg ttaaatttca gacatttaag 120

gcagatttgt tgatttcaaa atacgcacca aaggcagaat accaaaaaat tacaacagga 180

gagtcagatt ctcagctcga aatgactcgt cgtttggaat tgagctttga agagtatctt 240

gatttgcgtg attactgtct tgaaaaggga gttgatgtgt tttcgacacc ttttgatgag 300

gaatcattgg acttcttgat tagcacagat atgcccgttt ataagattcc atctggtgag 360

attaccaatc ttccctattt ggaaaaaatt ggtcgtcaag ctaagaaagt tattctttca 420

actggtatgg ctgttatgga tgaaattcat caagcggtga agattttgca ggaaaatgga 480

acgaccgata tttcgatttt gcattgtaca accgagtatc caacccctta ccctgctttg 540

aatttgaatg tcttgcatac cttgaaaaaa gaatttccaa acttaacaat tggctattca 600

gaccatagtg ttggttcaga agtacccatc gctgctgcag caatgggagc tgaattgatt 660

gaaaagcact ttactctgga caatgaaatg gaaggaccag atcataaagc gagtgctact 720

cctgatatct tagcagcctt ggtaaaagga gtgaggatag tggaacaatc tcttggtaaa 780

tttgaaaaag agccagaaga agttgaagta cgaaataaaa ttgtagctag aaaatctatt 840

gttgccaaaa aagcaattgc taaaggcgaa gtctttacag aagaaaacat cactgtcaaa 900

agaccaggaa atggaatttc gccaatggaa tggtacaaag tcttggggca ggtgagtgag 960

caggattttg aggaagacca aaatatttgc catagtgctt ttgaaaatca aatgtaa 1017

<210> 147
<211> 338
<212> PRT
<213> Streptococcus suis

<400> 147

Met Val Tyr Ile Ile Ala Glu Ile Gly Cys Asn His Asn Gly Asp Val
1 5 10 15

His Leu Ala Arg Lys Met Val Glu Val Ala Val Asp Cys Gly Val Asp
20 25 30

Ala Val Lys Phe Gln Thr Phe Lys Ala Asp Leu Leu Ile Ser Lys Tyr
35 40 45

Ala Pro Lys Ala Glu Tyr Gln Lys Ile Thr Thr Gly Glu Ser Asp Ser
50 55 60

Gln Leu Glu Met Thr Arg Arg Leu Glu Leu Ser Phe Glu Glu Tyr Leu
65 70 75 80

Asp Leu Arg Asp Tyr Cys Leu Glu Lys Gly Val Asp Val Phe Ser Thr
85 90 95

Pro Phe Asp Glu Glu Ser Leu Asp Phe Leu Ile Ser Thr Asp Met Pro
100 105 110

Val Tyr Lys Ile Pro Ser Gly Glu Ile Thr Asn Leu Pro Tyr Leu Glu
115 120 125

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Lys Ile Gly Arg Gln Ala Lys Lys Val Ile Leu Ser Thr Gly Met Ala
    130                 135                 140

Val Met Asp Glu Ile His Gln Ala Val Lys Ile Leu Gln Glu Asn Gly
145                 150                 155                 160

Thr Thr Asp Ile Ser Ile Leu His Cys Thr Thr Glu Tyr Pro Thr Pro
                165                 170                 175

Tyr Pro Ala Leu Asn Leu Asn Val Leu His Thr Leu Lys Lys Glu Phe
            180                 185                 190

Pro Asn Leu Thr Ile Gly Tyr Ser Asp His Ser Val Gly Ser Glu Val
        195                 200                 205

Pro Ile Ala Ala Ala Ala Met Gly Ala Glu Leu Ile Glu Lys His Phe
    210                 215                 220

Thr Leu Asp Asn Glu Met Glu Gly Pro Asp His Lys Ala Ser Ala Thr
225                 230                 235                 240

Pro Asp Ile Leu Ala Ala Leu Val Lys Gly Val Arg Ile Val Glu Gln
                245                 250                 255

Ser Leu Gly Lys Phe Glu Lys Glu Pro Glu Glu Val Glu Val Arg Asn
            260                 265                 270

Lys Ile Val Ala Arg Lys Ser Ile Val Ala Lys Lys Ala Ile Ala Lys
        275                 280                 285

Gly Glu Val Phe Thr Glu Glu Asn Ile Thr Val Lys Arg Pro Gly Asn
    290                 295                 300

Gly Ile Ser Pro Met Glu Trp Tyr Lys Val Leu Gly Gln Val Ser Glu
305                 310                 315                 320

Gln Asp Phe Glu Glu Asp Gln Asn Ile Cys His Ser Ala Phe Glu Asn
                325                 330                 335

Gln Met
```

<210> 148
<211> 1398
<212> DNA
<213> Streptococcus suis

<400> 148
atgaaaaaag ttagttttg gttgggaatc aacatcgcct tgctcggcat aatggtaagt 60

ttggctgtat ggttatttgc aggacttcaa gaaagacagg ttagtcagtt tattgaggaa 120

aaacaacaaa ccatcctcgc taaaggcaag ggcaaaattc aagagggaaa tattgatacg 180

acccatgttg tggctgcctt acctacagat gatgcaggtc atgtcttagg acctgtagaa 240

agtcgaatga tttcctatgt tcaaagacgc tttggtcata aaaaaccagc aggaaaaatt 300

caaaaactgg tctttgtttc atcaatagag ggaaaaacaa attttaaaaa tgtaacagct 360

cgtgaaattc aagcggagca ctataaagta gacaacttgc aaattaaaaa gcaggataaa 420

ctcccgtctg agcgtgtgtt attgacgcag gacaatgaat tgtttactct agaagattta 480

ttacctaatt tatcctctgc agcaagtatt attgttgatc atctaagaga agccttactt 540

gctcaaggaa tgaaagagac tgatgtagaa gcgattgtca aaaaatttga aacactagac 600

ttaaatgcta tttcctttag ttatggagac agccaattaa ccttacaatt accagatggt 660

tacggcatca accagttggt tctaccaatt tcagatttgt acccagtagt gaagagtgat 720

tatctagtag atgctgataa ggttggctat gatgagtata tggctgcaca agttgtggat 780

aagaaacatt tgagacaggt cgctctaacc tttgatgatg gacccaatcc gaacacaaca 840

ccggtagttt tagatttact gaaaaaatat aatgccaaag caactttctt tgttgttggt 900

aaggctgtag ttgggaatga agctattctc cgtagaatgg tagcagaagg gcacgtcatc 960

gccaaccata cgtggaatca tcccaatcta gtcactattt ctggggagca agtacagaga 1020

gaaattcaag atacacaggc agctattact gaagctacag gtattgtacc aacaatggtc 1080

cgtcctcctt atggttctgt taaccaggcc gttgtcaatc agatgggact tccatcgatt 1140

tattggtcgg tcaattctaa agattggaag agtcgcaatc cccaagccat tttgaaagaa 1200

ataaaagaac agacttgtcc aggaagtatt atcctaatgc atgatattca tcagtcaaca 1260

gttgatagcc tcgagtctgt attgcaatat ttaacaggtg agggctacaa tatggttact 1320

gtaacagact tactcgctag tcctctcaat cctcaattaa tctattattc acaagaatta 1380

tccggcccag cccagtaa 1398

<210> 149
<211> 465
<212> PRT
<213> Streptococcus suis

<400> 149

Met Lys Lys Val Ser Phe Trp Leu Gly Ile Asn Ile Ala Leu Leu Gly
1 5 10 15

```
Ile Met Val Ser Leu Ala Val Trp Leu Phe Ala Gly Leu Gln Glu Arg
            20                  25                  30

Gln Val Ser Gln Phe Ile Glu Glu Lys Gln Gln Thr Ile Leu Ala Lys
        35                  40                  45

Gly Lys Gly Lys Ile Gln Glu Gly Asn Ile Asp Thr Thr His Val Val
    50                  55                  60

Ala Ala Leu Pro Thr Asp Asp Ala Gly His Val Leu Gly Pro Val Glu
65                  70                  75                  80

Ser Arg Met Ile Ser Tyr Val Gln Arg Arg Phe Gly His Lys Lys Pro
                85                  90                  95

Ala Gly Lys Ile Gln Lys Leu Val Phe Val Ser Ser Ile Glu Gly Lys
            100                 105                 110

Thr Asn Phe Lys Asn Val Thr Ala Arg Glu Ile Gln Ala Glu His Tyr
        115                 120                 125

Lys Val Asp Asn Leu Gln Ile Lys Lys Gln Asp Lys Leu Pro Ser Glu
    130                 135                 140

Arg Val Leu Leu Thr Gln Asp Asn Glu Leu Phe Thr Leu Glu Asp Leu
145                 150                 155                 160

Leu Pro Asn Leu Ser Ser Ala Ala Ser Ile Ile Val Asp His Leu Arg
                165                 170                 175

Glu Ala Leu Leu Ala Gln Gly Met Lys Glu Thr Asp Val Glu Ala Ile
            180                 185                 190

Val Lys Lys Phe Glu Thr Leu Asp Leu Asn Ala Ile Ser Phe Ser Tyr
        195                 200                 205

Gly Asp Ser Gln Leu Thr Leu Gln Leu Pro Asp Gly Tyr Gly Ile Asn
    210                 215                 220

Gln Leu Val Leu Pro Ile Ser Asp Leu Tyr Pro Val Val Lys Ser Asp
225                 230                 235                 240

Tyr Leu Val Asp Ala Asp Lys Val Gly Tyr Asp Glu Tyr Met Ala Ala
                245                 250                 255

Gln Val Val Asp Lys Lys His Leu Arg Gln Val Ala Leu Thr Phe Asp
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            260                 265                 270

Asp Gly Pro Asn Pro Asn Thr Thr Pro Val Val Leu Asp Leu Leu Lys
        275                 280                 285

Lys Tyr Asn Ala Lys Ala Thr Phe Phe Val Val Gly Lys Ala Val Val
    290                 295                 300

Gly Asn Glu Ala Ile Leu Arg Arg Met Val Ala Glu Gly His Val Ile
305                 310                 315                 320

Ala Asn His Thr Trp Asn His Pro Asn Leu Val Thr Ile Ser Gly Glu
                325                 330                 335

Gln Val Gln Arg Glu Ile Gln Asp Thr Gln Ala Ala Ile Thr Glu Ala
            340                 345                 350

Thr Gly Ile Val Pro Thr Met Val Arg Pro Pro Tyr Gly Ser Val Asn
        355                 360                 365

Gln Ala Val Val Asn Gln Met Gly Leu Pro Ser Ile Tyr Trp Ser Val
    370                 375                 380

Asn Ser Lys Asp Trp Lys Ser Arg Asn Pro Gln Ala Ile Leu Lys Glu
385                 390                 395                 400

Ile Lys Glu Gln Thr Cys Pro Gly Ser Ile Ile Leu Met His Asp Ile
                405                 410                 415

His Gln Ser Thr Val Asp Ser Leu Glu Ser Val Leu Gln Tyr Leu Thr
            420                 425                 430

Gly Glu Gly Tyr Asn Met Val Thr Val Thr Asp Leu Leu Ala Ser Pro
        435                 440                 445

Leu Asn Pro Gln Leu Ile Tyr Tyr Ser Gln Glu Leu Ser Gly Pro Ala
    450                 455                 460

Gln
465
```

<210> 150
<211> 741
<212> DNA
<213> Streptococcus suis

<400> 150

atggctcaat taaaaatcaa tgtccatgac ttacacaagt cttatggtaa aaacgaggtc 60

ctcaaaggta ttactgctaa gttctatgaa ggcgatgttg tttgtatcat cggtccttct 120

ggttcaggta aatcgacctt ccttcgtaca atgaacttac ttgaaaccat tacaagtggt 180

caagtaacag tcgatggata tgacttaacc gaccctaaga cagatgttga cgccttccgt 240

tcaaatgtcg gaatggtatt ccaacacttc aacctcttcc ctcatatgtc tgtacttgac 300

aatatcacat ttgcgccaat cgaacacaaa ttgatggaca agtctgaagc tgaaaaggtc 360

ggcatggaat tattggaaaa agttggattg gcagacaaac gcgacgctat gccagacagc 420

ctttcaggtg gtcagaagca acgtgttgct atcgctcgtg ccctcgctat gaacccagac 480

atcatgctct tcgacgaacc aacttctgcc cttgaccctg aaatggttgg cgatgtactc 540

aacgttatga aagacttggc tgagcaaggt atgaccatgt taatcgtcac acacgagatg 600

ggctttgccc gcaaggttgc taatcgcgtt atcttcactg atggcggtga gttccttgaa 660

gatggtacac cagagcaaat ctttgataac ccacaacatc cacgtttaca agacttcctt 720

gataaggtct tgaacgtcta a 741

<210> 151
<211> 246
<212> PRT
<213> Streptococcus suis

<400> 151

Met Ala Gln Leu Lys Ile Asn Val His Asp Leu His Lys Ser Tyr Gly
1 5 10 15

Lys Asn Glu Val Leu Lys Gly Ile Thr Ala Lys Phe Tyr Glu Gly Asp
20 25 30

Val Val Cys Ile Ile Gly Pro Ser Gly Ser Gly Lys Ser Thr Phe Leu
35 40 45

Arg Thr Met Asn Leu Leu Glu Thr Ile Thr Ser Gly Gln Val Thr Val
50 55 60

Asp Gly Tyr Asp Leu Thr Asp Pro Lys Thr Asp Val Asp Ala Phe Arg
65 70 75 80

Ser Asn Val Gly Met Val Phe Gln His Phe Asn Leu Phe Pro His Met
85 90 95

Ser Val Leu Asp Asn Ile Thr Phe Ala Pro Ile Glu His Lys Leu Met
100 105 110

Asp Lys Ser Glu Ala Glu Lys Val Gly Met Glu Leu Leu Glu Lys Val
115 120 125

Gly Leu Ala Asp Lys Arg Asp Ala Met Pro Asp Ser Leu Ser Gly Gly
130 135 140

Gln Lys Gln Arg Val Ala Ile Ala Arg Ala Leu Ala Met Asn Pro Asp
145 150 155 160

Ile Met Leu Phe Asp Glu Pro Thr Ser Ala Leu Asp Pro Glu Met Val
165 170 175

Gly Asp Val Leu Asn Val Met Lys Asp Leu Ala Glu Gln Gly Met Thr
180 185 190

Met Leu Ile Val Thr His Glu Met Gly Phe Ala Arg Lys Val Ala Asn
195 200 205

Arg Val Ile Phe Thr Asp Gly Gly Glu Phe Leu Glu Asp Gly Thr Pro
210 215 220

Glu Gln Ile Phe Asp Asn Pro Gln His Pro Arg Leu Gln Asp Phe Leu
225 230 235 240

Asp Lys Val Leu Asn Val
245

<210> 152
<211> 1038
<212> DNA
<213> Streptococcus suis

<400> 152
atgaaacaag cagtagcagt tatttttggg acctttgctc ccatgcacaa gggtcatatt 60

gacctgattc aacgagccaa gcgggaatgc gatcgggcgg tcgtcatcgt gtcgggctat 120

aaaaatgacc gtggtcatca gattggtctc ggtttgcaga aacgtttccg ctatatccgc 180

gaaaccttca acgacgagcc tctggtatcc gtctttaagc tagacgagga gggaatgccc 240

ccttatccag aaggttgggc accttggtta gaagccttgc aggccttggt ggcgattaag 300

gagaacgaag agctcgtctt ctacgtttcg gagcaggaat atgccgaaga gttgcgttct 360

cgtggtttca aggcttcctt tacagagcga aattttggca tttcagccac tcttatccgc 420

gagcagtcgg ctaagtattg gaatatgatt gccaagcctt tccgccgtca tttttctaag 480

aatatcttgg tggttggctc tgcttcaaat ggtaaaacca cgctagtgcg ggatttgggg 540

cgttattatt cctgtcctgt ttcgctggaa tatgcccgct actaccagca acgttacaat 600

gtgcgtgatg atgagttgac aggcaaggac tacaactatc tcttgacggg ccagtatcgc 660

cagacctcag acctgattga ctcggatacc aatcgtggtc tggtcattgc ggacaccaac 720

gcgaccgtga cggaagccta ctacgactac tacatcggcg aaacttctag ttctttccac 780

tcgctctgtg ctgatacggt taagaatgaa aaatgggacc tgattatctt tgttctaccg 840

acaggctctt atgtggatga cggttttcgg gatatgacaa tggctgacgc agaaattcgc 900

aatgccttta ccgaacattt gaaagaactg gttgctaaaa atcacccgca ggcttcgctg 960

gcctttatcg gtgggtctta tgcagaaaat tatgccaagg ctatcgagct aatcgatgct 1020

atctatcagg aattttag 1038

<210> 153
<211> 345
<212> PRT
<213> Streptococcus suis

<400> 153

Met Lys Gln Ala Val Ala Val Ile Phe Gly Thr Phe Ala Pro Met His
1 5 10 15

Lys Gly His Ile Asp Leu Ile Gln Arg Ala Lys Arg Glu Cys Asp Arg
20 25 30

Ala Val Val Ile Val Ser Gly Tyr Lys Asn Asp Arg Gly His Gln Ile
35 40 45

Gly Leu Gly Leu Gln Lys Arg Phe Arg Tyr Ile Arg Glu Thr Phe Asn
50 55 60

Asp Glu Pro Leu Val Ser Val Phe Lys Leu Asp Glu Glu Gly Met Pro
65 70 75 80

Pro Tyr Pro Glu Gly Trp Ala Pro Trp Leu Glu Ala Leu Gln Ala Leu
85 90 95

Val Ala Ile Lys Glu Asn Glu Glu Leu Val Phe Tyr Val Ser Glu Gln
100 105 110

Glu Tyr Ala Glu Glu Leu Arg Ser Arg Gly Phe Lys Ala Ser Phe Thr
115 120 125

Glu Arg Asn Phe Gly Ile Ser Ala Thr Leu Ile Arg Glu Gln Ser Ala
130 135 140

```
Lys Tyr Trp Asn Met Ile Ala Lys Pro Phe Arg Arg His Phe Ser Lys
145                 150                 155                 160

Asn Ile Leu Val Val Gly Ser Ala Ser Asn Gly Lys Thr Thr Leu Val
                165                 170                 175

Arg Asp Leu Gly Arg Tyr Tyr Ser Cys Pro Val Ser Leu Glu Tyr Ala
            180                 185                 190

Arg Tyr Tyr Gln Gln Arg Tyr Asn Val Arg Asp Asp Glu Leu Thr Gly
        195                 200                 205

Lys Asp Tyr Asn Tyr Leu Leu Thr Gly Gln Tyr Arg Gln Thr Ser Asp
    210                 215                 220

Leu Ile Asp Ser Asp Thr Asn Arg Gly Leu Val Ile Ala Asp Thr Asn
225                 230                 235                 240

Ala Thr Val Thr Glu Ala Tyr Tyr Asp Tyr Tyr Ile Gly Glu Thr Ser
                245                 250                 255

Ser Ser Phe His Ser Leu Cys Ala Asp Thr Val Lys Asn Glu Lys Trp
            260                 265                 270

Asp Leu Ile Ile Phe Val Leu Pro Thr Gly Ser Tyr Val Asp Asp Gly
        275                 280                 285

Phe Arg Asp Met Thr Met Ala Asp Ala Glu Ile Arg Asn Ala Phe Thr
    290                 295                 300

Glu His Leu Lys Glu Leu Val Ala Lys Asn His Pro Gln Ala Ser Leu
305                 310                 315                 320

Ala Phe Ile Gly Gly Ser Tyr Ala Glu Asn Tyr Ala Lys Ala Ile Glu
                325                 330                 335

Leu Ile Asp Ala Ile Tyr Gln Glu Phe
            340                 345
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