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(71) Applicant (for all designated States except US): **Universidad de Córdoba** [ES/ES]; Universidad de Córdoba Rectorado, Avda. Medina Azahara, E-14071 Córdoba (ES).

(72) Inventor; and

(71) Applicant : **BENINATI, Concetta** [IT/IT]; Via Consolare Pompea 437, I-98167 Messina (IT).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **Rodríguez Ortega, Manuel José** [ES/ES]; Departamento de Bioquímica y Biología, Molecular, Edificio "Severo Ochoa", 1ª planta, Campus de Rabanales, Universidad de Córdoba E-14071 Córdoba (ES).

(74) Agents: **SPADARO, Marco** et al.; Studio Associato Leone E Spadaro, Viale Europa 15, I-00144 Roma (IT).

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(54) Title: IMMUNOGENIC AND THERAPEUTIC COMPOSITIONS FOR STREPTOCOCCUS SUIIS

(57) Abstract: The present invention refers to protein Ssu05_0473 and protein Ssu05_0272 or immunogenic derivatives thereof for use as a medicament, in particular for use as a medicament against Streptococcus suis colonization or infection in humans and animals. The present invention also refers to a vaccine comprising at least one of said proteins or immunogenic derivatives thereof, to a method for the diagnosis of bacterial infections using said proteins or immunogenic derivatives thereof and to a kit for the diagnosis of Streptococcus suis infections.



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IMMUNOGENIC AND THERAPEUTIC COMPOSITIONS FOR STREPTOCOCCUS SUIS

Technical Field

The present invention refers to the field of infectious diseases and more in particular it refers to protein Ssu05_0473 and protein Ssu05_0272 or immunogenic derivatives (also referred to as polypeptide or polypeptides of the invention) thereof for use as a medicament, in particular for use as a medicament against *Streptococcus suis* colonization or infection in humans and animals.

The present invention also refers to a vaccine comprising at least one of said proteins or immunogenic derivatives thereof, to a method for the diagnosis of bacterial infections using said proteins or immunogenic derivatives thereof and to a kit for the diagnosis of *Streptococcus suis* infections.

Background of the invention

Streptococcus suis is an important pathogen associated with a wide range of diseases in pigs, including meningitis, septicaemia, pneumonia, endocarditis, and arthritis. *S. suis* can be transmitted to human beings by direct contact (Lun et al, 2007). Based on the capsular polysaccharides, 35 serotypes have been identified (types 1–34 and 1/2).

The resurgence of infectious diseases caused by *Streptococcus suis* has heightened public concern, largely because resistance to certain antibiotics has recently emerged. In July 2005, a large outbreak of human *S. suis* infection occurred in Sichuan province, China, accompanied by several sporadic cases in other provinces. This was the third reported outbreak of human *S. suis* infections that has occurred in China, with two earlier outbreaks occurring in Jiangsu province in 1998 and 1999. In the past 8 years in China, at least 237 people have been infected with *S. suis* and 53 of them died. The repeated intensive outbreaks of human *S. suis* infection have raised great public

concern worldwide regarding it as an emerging zoonotic agent. Therefore, research on other treatments, mainly vaccines, are key to overcome drug resistance.

To date, several approaches have been applied to develop vaccines against this pathogen. Nevertheless, the success achieved has been very limited due to serotype-
5 or strain-dependent protection. Using the capsular polysaccharide as an antigen, the results have been unsatisfactory due to poor immunogenicity. Then, interest has been focused on surface proteins as potential vaccine candidates. Some of them have been proposed and tested, as sulysin, muramidase-released protein (MRP) or extracellular
10 protein factor, but their use is limited because an important number of virulent strains in some geographical regions do not express these proteins (Li et al, 2006 and 2007). More recently, protein Sao has been proposed as a new protein candidate (Li et al, 2006 and 2007).

There is a need of new vaccine compositions based on new antigens, or fragments thereof, capable of recognising a higher number of serotypes and induce immune
15 response in animals or humans infected by virulent strains of such serotypes.

During the last years, one of the inventors and colleagues have set up and applied a novel proteomics-based technology for the massive identification of surface protein antigens in several Gram-positive pathogenic bacteria (Rodríguez-Ortega et al 2006, Nat. Biotechnol. 24 (2): 191-197; Rodríguez-Ortega et al 2008, BMC Genomics 9: art
20 588). In 2007, the present inventors focused their attention on the identification of surface proteins from *Streptococcus suis* using the previously cited proteomic strategy. For this microorganism, two genomes have been very recently sequenced and annotated, those corresponding to strains 05ZYH33 and 98HAH33 (Chen et al, PLoS ONE 2007, 2(3): e315).

25 The method for the identification of antigens or their fragments of *Streptococcus suis* surface proteins, by means of identification of peptides generated after protease digestion of their surface-exposed domains, comprises the following steps:

- a) Bacterial culture in Todd-Hewitt Broth until mid-exponential phase is reached;
 - b) Centrifugation, discarding of the culture medium (supernatant) and washing of bacteria (pellet) three times with PBS;
 - c) Bacterial resuspension in isotonic buffer, which contains the protease;
 - 5 d) Digestion at 37 °C for 30 minutes, with very gentle shaking;
 - e) Centrifugation to recover the supernatant, which contains the released peptides;
 - f) Sample cleaning/desalting by employing reversed-phase chromatography cartridges, and
 - g) Separation and identification of peptides by 2-D LC/MS/MS.
- 10 Briefly, such a strategy consists of incubating live cells with proteases (trypsin, proteinase K) in order to cleave the exposed domains of surface proteins. The peptides generated from shaved bacterial cells are recovered and then analysed by two-dimensional liquid chromatography coupled to tandem mass spectrometry (2-D LC/MS/MS). The mass spectra thus obtained are used for database searching and
- 15 peptide sequence identification. This approach allows the recovery of a fraction of surface proteins in a fast and reliable way without cytoplasmic contamination, as cells remain intact. Then, false positives are discarded. Moreover, the identified proteins are contrasted by computational analysis and their sequences are inspected to correct possible errors in function prediction.
- 20 Surface-exposed *Streptococcus suis* antigens as defined below can be identified using a simple and cost-effective approach which combines liquid chromatography and tandem mass spectrometry. The surface-exposed *S. suis* antigens can be used as active agents in compositions for preventing and for treating *S. suis* infections.

Disclosure of Invention

- 25 By combining bioinformatic analysis, mainly algorithms for subcellular location prediction, and experimental work, i.e. the above cited proteomic approach, the inventors of the present invention have found 3 proteins for which the predicted

(cytoplasmic) functions did not correspond neither to protein sequence features, determined by bioinformatic analysis, as the presence of cell-wall anchoring LPXTG (SEQ ID NO: 1) motifs, nor to surface localisation according to proteomics identification, which can be also corroborated by FACS analysis.

- 5 It has been then found that the predicted and primarily annotated functions of these 3 proteins were wrong.

In particular it has been found that two of these 3 proteins, Protein Ssu05_0473 and protein Ssu05_0272, and their fragments are immunogenic and show a clear protective activity against *S. suis* infection.

- 10 Therefore, object of the present invention are proteins Ssu05_0473 and Ssu05_0272 or immunogenic derivatives thereof, in particular the fragment aa853-aa1252 of protein Ssu05_0473, for use as a medicament.

The polynucleotide sequence (SEQ ID NO: 2) of the whole gene coding for Ssu05_0473 is:

15 gtgcaagaaacaccagtaacaacggaattagtaaattaacagaaccgatggtgacaact
cagcaaactccgatagtgggtagctctgctgatgaagttcaatctcctgcgacagaaacg
gttctgaccctgctgagcaagtgtcaacagagtctgctagcaaaccagaccgaccaa
gaacggattactgagccaactaccatagttcacaaaacgaatgattatgagggtgactgca
acatttgatgcgagtgctcaatttcccaaagatggtgagtttaatgtagagaactagat
20 agccaatcagaagaatatcggacacattatgaaaaaccaaggagaagcttggagtaaaa
aaacttgatatgcccgattctttgatattcatttttcatcacagggccaagagggtcgag
cctgcctcccctgtgaagattacgattagaacaaggaattagttcaacggccagatgat
agtaaactgaaaattgtccactttgaagagaatagccagatagaagttttagaggccgaa
acgaaggaaaatgaacaacaatctccgagatcagttttgacgctagccatttttctggt
25 tatggagatgttttagcaaaactattatactgtcaaatttacctacactgacacagcaggt
caagaacaagtcattacgagtttgattgataagactgtggggacttcacttggtagtttg
cctgaagctccttttaaaaacggttatgtattttctcattggattaataaagatactggt

gatcggattgatgaaaatacgggtgataactggcgacctgactgctgtagcagtatTTAAT
aatatcaatatctacaccattgatgtcacgtatTTCTaccacaatgTTTCTAAAAACCA
gatattacgattgataaggaaacgggtgcaactggaagcaagtgatgctccatatcaactg
acaccacctgcttctactgaggtgagcaacaagaggatacaactTTaccagcagatgct
5 atttactaccctgaaaaaccacttcttgagTTTtacagcagatcaactagagcgactggac
gtagcagatggagtgatgataattatattagcgaaagagtacaatatgTTccttacaat
gcagagtatgatgtcatttataggctaaagaatTTgacggggtcaggttatttctgatatc
cagactgTTcatgtccgtggggTactgggttcaactgTTacaccgcaaattCTaacctac
aactatgctgactTTTgaacgtgctgatacagcaaagattgagaatactagcggccAAAA
10 ttgtatgTTTattatacacggaaaaccttactCTTTcttataattCCAatggcggtagt
aatattccccacaacagggTctatacगतtagccagattaagattTTctacaactactcca
acaaaagaggggtatacattTgaaggTTggtatgataatcctggactgactggaaacaag
attacaggctctctcacctTaaagaaagatacaactTTTgtatgCGaaatggacaccgaac
agggttcgctacatcattgggtattacaaggaagtctatgacaacaacactggaacaacc
15 tcctatgtatatगतtagttcggcagctggTTggggTctggTTgggactactgTaaaagga
acagattcaaatattggacctcctccgccaacccaacaaccattggcagcattggctac
gagaaggatgTTactaaaagtgctgctTcaagtattgaaattgcagctgatggaacatct
gTTTTgaaagTTTactataaactgaagcgctacactTTTgaattTTTctTaaatgataac
aatGCCaatatcaccataaatggtaagacctactctगतtaggaataggTacatगतacgt
20 gatgTTgtgctTggTcaagatgtctcaaagTTatggccagcttctataacaaatcctaaa
gaagtatacगतgataaagtctactatगतtagctatggtaggccgataagatactTTTTT
gactactggattTTTTgggaatctactctTcaaaacagatcggTtcgaagTTgTTgagtct
atgTTGCCaacgagtggaaacaaccagTaaagTTcacagcatattggaataccaaggTaca
caggctaacgTTgaatattggTTacaacaaccगतggttcttataagaagtctगतata
25 tatagtcaatctTTcctaaagactgcaggactgctaccgaaagaaatTTTTgggatattct
tattatacगतgctagTTTTactgaagcaggcattaggTattacggaaactcaaggaaat
acatatcgTTTctactataaacGCCaagcTTTcaaaattgactattattacaatagtact
gggTtagattcaaagagTaaacattctctTTgagagTaaacatcaatacagcaactTataat
taccaaccaccagTTctactggagTTgatgctgattatacatggggTggatggTataca
30 gattcgTTattgacagagaagtataactTTaataagatgcctGCCaataactTggTTctc

tatgctaagtggattgctccaaaatttaaggttacttttgatatcaacgggtggtaatgga
actgcccctgctcgatcaaagtgttgaaaaatacaaatcgcctaaggcccctgctgatcca
acgagggaaacattacaatctccttggttggtctacagaagatgggaaactctatagttgg
agcgaccaagtaaccaaggatgtcaaattgactgcgaagtgggaattgaaaccattaact
5 tatactgttcgctatcttgaagcaggtacaaatacgcgaattggctgctgataaagtgatt
acgagtccagcgccttgattgaatcaagctatcacagaaaaggcattgggaattacaggt
tatcgaccagataagagtagtcaaacaattaacctgaatcttagagggaaacgaaataact
ttctactatacaccgaaggtaaaagatggtgtttacaccatcaagtatgtcttgaaggat
aaccagctattgaggtagcacctacagttactcgttaaggttgatggaagtatcatccgt
10 gcgaaagaagctgctgcagatgtgaataaacagcatttgcagagtcagtcctggtgttact
gctgaaatgctggaaatggattactatccaactgtaaatactcaaagtttgattctaact
tctagtgatgcaaataatgtgattgtttttgaaatgctcggattcgatactcaaaccctt
acggtaaattatcttgatattggatggtaagaaaatcgcaggtcaagaaccattggtagtt
taccgaaagaaaccaggtagtttctggttagatcataagactataaatggttatacctat
15 gatcattcgttagacagtgagaatgcagctgataagacagtataccgtgtaagtaagggt
ggtcacataaccattgatctctattataagaaaaacctaacactgaccgcattagataag
tccaaagtttatgatggcactgccctatcaagttcgggtctgtctgatttaaactccttcg
tacagttcagaactgaaaacaggtgacaaattggttgccatcagctatgatggaagtcaa
acaaatgctggtagtagcgcaacgacacctaacttgctaacatcagcaatgcatctggt
20 gctgaccggacgaatctctatcacataacctatcaaccgggtaacttaagagttgaccaa
cagccagttgtggttgtcatcgacggtgaaaagaaatcgaaaatctatgatggcaaagca
gaagcagttggttataaggtgacggaataagagattcaagcgggtctctataaaaaaaca
gatatcaaatttaatggttcaagttcagagcagtcggttaagaaaaaagatgcaggaatt
tatccactccttttaagtaatagattctcaaataccaatcagaatctccaagtagatttc
25 ttgattctctgatggggaattaagaatagatagacggcaactgaccataacatctgactcc
gctagcaaggcttatgacggtagcgagcttcgtgctgacaatatcacagtctctgtacct
gctggtgttgactacacaggttttctcgatggcgaagggtattataccaaccttcttaggc
ggtccaatcgattctggaatgatgccaaacttctttgaaatgctctaaaatctggcacg
aatttagctaattatgagttagatatccagtttggccagctcaagggtcaaagaagtcac
30 aagattcagaaaacagacctggcctggcaggccctttcaggtggtaagtttgaattgacc

aagtgggatggtttgaactgggctcaggtagatgggggtgcaggaatttgccattaccagc
 aaagatgggtattcggataccggtaggcttagaagcaggtcgcctaccgtctgcaagaattg
 gcagcacctgatgggttttatcgtcttgatagctatatctacttcagcatcaaggaaatc
 tttaacgaagataagacatcttccttctacacagtttccttgtctgatgaagcaggcaag
 5 gatgctagtcggaacgagcaacgcttaccaaggttagtggtgatgccagtcacgcac
 caagtggccaatgagcagggcagagccttgccaagcacagggcggagatggacagaagtgg
 tttattctttctggccttgtcctcatagccatctcctatctgatgcacatctgtttgttcaa
 cgtcgaaggtaa

The amino acid sequence (SEQ ID NO: 3) coded by the above gene is:

10 MQETPVTEISKLTEPMVTTQQTPIVGSSADEVQSPATETVPDPAEQVSTESASKPQTDQERITEPTTIV
 HKTNDYEV TATFDASAQFPKDVEFNVRELD SQSEYRTHYEKTKEKLGVKKLVYARFFDIHFSSQGQVEE
 PASPVKITIRNKELVQRDDSKLKIVHFEENSQIEVLEAETKENEQQISEISFDASHFSVYGDVLANYYT
 VKFTYTDTAGQEQVITSLIDKTVGTVSLGSLPEAPFKNGYVFSHWINKDTGDRIDENTVITGDLTAVAVFN
 NINIYTIDVTFYHNVSKNQDITIDKETVQLEASDAPYQLTPPASTEVS NKEDTTLPADAIYYPEKPLLE
 15 FTADQLERLDVADGVDDNYISERVQYVPYNAEYDVIYRLKNLTGSGYSDIQTVHVRGVLGSTVTPQILTY
 NYADFERADTAKIENTSGQKLYVYYTRKFTLSYNSNGGSNIPQQGLYDSQIKISTTTPTKEGYTFEGW
 YDNPGLTGNKITGSLTLKKDTTLYAKWTPNRVRYIIGYYKEVDNNTGTTSYVYDSSAAGWGLVGTTVKG
 TDSNIGPPPPTQTIGSIGYEKDVTKSAASSIEIAADGTSVLKVYYKLRKRYTFEFLNDNNANITINGKT
 YSDRNRYMIRDVVLGQDVSKLWPASITNPKEVYDDKVYYDSYGRPIRYFFDYWIFGNLLFKTDRFEVVES
 20 MLPTSGTTSKFTAYWNTQGTQANVEYW LQQPDGSYKKS DIYSQSFLKTAGLLPKEIFGYSYYTDASFTEA
 GIRYYGTQGNTYRFYYKRQAFKIDYYNSTGLDSKSNILFESNINTATYNYQPPRSTGVDADYTWGGWYT

DSSLTEKYNFNKMPANNLVLYAKWIAPKFVTFDINGNGTAPVDQSVKEYKYAKAPADPTREHYNFLGW
 STEDGKLYSWSQVTKDVKLTAKWELKPLTYTVRYLEAGTNTQLAADKVITSPALVLNQAITKALGITG
 YRPDKSSQTINLNFRGNEITFYYPKVVDVYTIKYVLKDNPAIEVAPTVTRKVDGSIIRAKEAAADVNK
 QHLQSQSGVTAEMLEMDYYPTVNTQSLILTSSDANNVIVFEYVGFDTQTFTVNYLDMDGKKIDGQEPLVV
 5 YRKKPGSFVVDHKTINGYTYDHSLSSENAADKTVYRVSKGGHITIDLYYKKNLTLTALDKSKVYDGTALS
 SSGLSDLNPSYSSELKTGDKLVAI SYDGSQTNAGSSATTPKLANISNASGADRTNFYHITYQPGNLRVDQ
 QPVVVVIDGEKSKIYDGKAEAVGYKVTEIRDSSGLYKKTDIKFNGSSSEQSVKKKDAGIYPLLLSNRFS
 NTNQNFQVDFLISDGELRIDRRQLTITSDSASKAYDGSELRADNITVSVPAGVDYTGFDGEGIIPTFLG
 GPIDSGMMPNFFEYALKSGTNLANYELDIQFGQLKVKEVIKIQKTDLAWQALSGGKFELTKWDGLNWAQV

10 DGVQEFAITSKDGIRIPVGLEAGRYRLQELAAPDGFIVLDSYIYFSIKEIFNEDKTSSFYTVSLSDEAGK
 DASPERATLTKVSGDASHRIQVANEQGRALPSTGGDGQKWFILSGLVLI AISYLMHLFVQRRR

Two fragments of the above coding gene were cloned, called FA1 and FA3. The nucleotide sequence (SEQ ID NO: 4) corresponding to fragment FA1 is:

GGATCCGGGAGTCTGCTAGCAAACCCAGACCGACCAAGAACGGATTACTGAGCCAACACTACCATAG
 15 TTCACAAAACGAATGATTATGAGGTGACTGCAACATTTGATGCGAGTGCTCAATTTCCCAAAGATG
 TTGAGTTTAAATGTTAGAGAACTAGATAGCCAATCAGAAGAATATCGGACACATTATGAAAAACCA
 AGGAGAAGCTTGGAGTAAAAAACTTGTATATGCCCGATTCTTTGATATTCATTTTTTCATCACAGG
 GCCAAGAGGTCGAGCCTGCCTCCCCTGTGAAGATTACGATTAGAAACAAGGAATTAGTTCAACGGC
 CAGATGATAGTAACTGAAAATTGTCCACTTTGAAGAGAATAGCCAGATAGAAGTTTTAGAGGCCG
 20 AAACGAAGGAAAATGAACAACAAATCTCCGAGATCAGTTTTGACGCTAGCCATTTTTCTGTTTATG
 GAGATGTTTTAGCAAACACTATTATACTGTCAAATTTACCTACACTGACACAGCAGGTCAAGAACAAG
 TCATTACGAGTTTGATTGATAAGACTGTGGGGACTTCACTTGGTAGTTTGCCTGAAGCTCCTTTTA
 AAAACGGTTATGTATTTTTCTCATTGGATTAATAAAGATACTGGTGATCGGATTGATGAAAATACGG

TGATAACTGGCGACCTGACTGCTGTAGCAGTATTTAATAATATCAATATCTACACCATTGATGTCA
 CGTATTTCTACCACAATGTTTCTAAAAACCAAGATATTACGATTGATAAGGAAACGGTGCAACTGG
 AAGCAAGTGATGCTCCATATCAACTGACACCACCTGCTTCTACTGAGGTGAGCAACAAAGAGGATA
 CAACTTTACCAGCAGATGCTATTTACTACCCTGAAAAACCACTTCTTGAGTTTACAGCAGATCAAC
 5 TAGAGCGACTGGACGTAGCAGATGGAGTGGATGATAATTATATTAGCGAAAGAGTACAATATGTTT
 CTTACAATGCAGAGTATGATGTCATTTATAGGCTAAAGAATTTGACGGGGTCAGGTTATTCTGATA
 TCCAGACTGTTTCATGTCCGTGGGGTACTGGGTTCAACTGTTACACCGCAAATTC AACCTACA
 ATGCTGACTTTGAACGTGCTGATACAGCAAAGATTGAGAATACTAGCGGCCAAAAATTGTATGTTT
 ATTATACACGGAAAACCTTTgGCGGCCGC

10 The amino acid sequence (SEQ ID NO: 5) coded by the above nucleotide sequence is:

MKLKVTVNGTAYDVDVDVDKSHENPMGTILFGGGTGGAPAPAAGGAGAGKAGEGEIPAPLAGTVSK
 ILVKEGDTVKAGQTVLVLEAMKMETEINAPT DGKVEKVLVKERDAVQGGQGLIKIGDLELIEGREA
 SAGIRESASKPQTDQERITEPTTIVHKTNDYEV TATFDASAQFPKDVEFNVRELD SQSEEYRTHYE
 KTKEKLGVKLVYARFFDIHFSSQGEVEPASPVKITIRNKELVQRPDDSKLKIVHFEENSQIEVL
 15 EAETKENEQQISEISFDASHFSVYGDVLANYITVKFTYTD TAGQE QVITSLIDKTVGTS LGSLPEA
 PFKNGYVFSHWINKDTGDRIDENTVITGDLTAVAVFNNINIY TIDVTYFYHNVSKNQDITIDKETV
 QLEASDAPYQLTPPASTEVS NKEDTTL PADAIYYPEKPLLEFTADQLERLDVADGVDDNYISERVQ
 YVPYNAEYDVIYRLKNLTGSGYS DIQTVHVRGVLGSTVTPQILTYNYADFERADTAKIENTSGQKL
 YVYYTRKTFGGRDLVL

20 Theoretical pI/Mw: 4.70 / 60039.82. The molecular weight (MW) of the recombinant fragment determined by SDS-PAGE corresponded to that calculated from the sum of the amino acid residues (this is valid for the rest of recombinant fragments described in this application).

The nucleotide sequence (SEQ ID NO: 6) corresponding to fragment FA3 is:

25 GGATCCGGAATAAGATGCCTGCCAATAACTTGGTCTCTATGCTAAGTGGATTGCTCCAAAATTTA
 AGGTTACTTTTGATATCAACGGTGGTAATGGAAGTGGCCCTGTGCGATCAAAGTGTGAAAAATACA
 AATACGCTAAGGCCCTGCTGATCCAACGAGGGAACATTACAATTTCTTGGTTGGTCTACAGAAG

ATGGGAAACTCTATAGTTGGAGCGACCAAGTAACCAAGGATGTCAAATTGACTGCGAAGTGGGAAT
 TGAAACCATTAACCTATACTGTTTCGCTATCTTGAAGCAGGTACAAATACGCAATTGGCTGCTGATA
 AAGTGATTACGAGTCCAGCGCTTGTATTGAATCAAGCTATCACAGAAAAGGCATTGGGAATTACAG
 GTTATCGACCAGATAAGAGTAGTCAAACAATTAACCTGAATTTTAGAGGGAACGAAATAACTTTCT
 5 ACTATACACCGAAGGTAAAAGATGTTGTTTACACCATCAAGTATGTCTTGAAGGATAACCCAGCTA
 TTGAGGTAGCACCTACAGTTACTCGTAAGGTTGATGGAAGTATCATCCGTGCGAAAGAAGCTGCTG
 CAGATGTGAATAAACAGCATTTCAGAGTCAGTCTGGTGTACTGCTGAAATGCTGAAATGGATT
 ACTATCCAACCTGTAAATACTCAAAGTTTGATTCTAACTTCTAGTGATGCAAATAATGTGATTGTTT
 TTGAATATGTCGGATTTCGATACTCAAACCTTTACGGTAAATTATTTGGATATGGATGGTAAGAAAA
 10 TCGATGGTCAAGAACCATTGGTAGTTTACCGAAAGAAACCAGGTAGTTTCGTGGTAGATCATAAGA
 CTATAAATGGTTATACCTATGATCATTTCGTTAGACAGTGAGAATGCAGCTGATAAGACAGTATAACC
 GTGTAAGTAAGGGTGGTCACATAACCATTGATCTCTATTATAAGAAAAACCTAACACTGACCGCAT
 TAGATAAGTCCAAAGTTTATGATGGCACTGCCCTATCAAGTTCGGGTCTGTCTGATTTAAATCCTT
 CGTACAGTTCAGAACTGAAAACAGGTGACAAATTGGTTGCCATCAGCTATGATGGAAGTCAAACAA
 15 ATGCTGGTAGTAGCGCAACGACACCTAAACTTGCTAACATCAGCAATGCATCTGGTGCTGACCGGA
 CGAATTTCTATCACATAACCgGCGGCCGC

The amino acid sequence (SEQ ID NO: 7) coded by the above nucleotide sequence is:

MKLKVTVNGTAYDVDVDVDKSHENPMGTILFGGGTGGAPAPAAGGAGAGKAGEGEIPAPLAGTV
 SKILVKEGDTVKAGQTVLVLEAMKMETEINAPTDGKVEKVLVKERDAVQGGQGLIKIGDLELIE
 20 GREASAGIRNKMPANNLVLYAKWIAPKFKVTFDINGGNGTAPVDQSVEKYKYAKAPADPTREHY
 NFLGWSTEDGKLYSWSDQVTKDVKLTAKWELKPLTYTVRYLEAGTNTQLAADKVITSPALVLNQ
 AITEKALGITGYRPDKSSQTINLNFGRNEITFYYPKVVDVYTIKYVLKDNPAIEVAPTIVTRK
 VDGSIIRAKEAAADVKNQHLQSQSGVTAEMLEMDYYPTVNTQSLILTSSDANNVIVFEYVGFDT
 QTFTVNYLDMGKKIDGQEPLVVYRKKPGSFVVDHKTINGYTYDHSLDSENAADKTVYRVSKGG
 25 HITIDLYYKKNLTLTALDKSKVYDGTALSSSGLSDLNPSYSSELKTGDKLV AISYDGSQTNAGS
 SATTPKLANISNASGADRTNFYHITGGRDLVL

Theoretical pI/Mw: 6.07 / 58708.25

The polynucleotide sequence (SEQ ID NO: 8) of the whole gene coding for Ssu05_0272 is:

gtgggaaaatgtataaatctatatTTTTAagaaggagccaattatgagcaagcagaaagtt
gtgtccagtttattattaagcacagtcgtattagggggattagccttttattcaacgaca
5 acggttaaagcagaatcgctagaaccagatgttacatcagttaatgcaagcgatgctact
aataaaccggtagtagcaaatgaagagggaggcgactTTTTtagctgaagaggagcttgaa
gatgatgacactttggaagaagaattaaatgagaaagccgaagaggttactgagccatct
tcacctgaagcacttctccaacctcgtgcatgatgagtgattctgaaactagtgggatg
gaagaaattccgatgaatgatgaaccttcagataatacggaaagaaaaggtagagaagcaa
10 cagtcgccattaattcaaacctcaaatgcagattataaaaagtggtaaagatcaagagaaa
cttaggacatcagtatctataaatctggtgaaggcgggaagaaggtcagattcagtgga
gtaacttttgatacaagcgaatggtctTTTaatgttaaacaatgggggtgTTTTatt
cttccaaatggtttggacttaacaaagattggtgataataatcaacatgatataacagcc
tccttcctacggatattaatgattacagaaattctggtcaagaaaaatatcgTTTTctt
15 agtagtaaacaaggtcttgacaacgaaaatggTTTTaattctcaatggaactggtctgct
ggtcaagctaatcctagtgaaacagtaaacagttggaaatcaggcaatcgTTTTgtctaag
atTTatttcataaatcaaataacagataactaccgaattgacctatactTTaactgctaag
gtaactgaaccaaatacagcaatcattccctcttctggcagtgatgaagagctTTacgtat
acaaactctaagagtacagaggttacttcgctaggtgcacgtgagattacccttgagaaa
20 gaaaaaactctaccacctaaggagaatccaaaaccagagccagaagctccaaaaccagac
gctccacaagcgccatcagcaccggaatctccaacggaagaacctaaagaaggaagacgct
ccagcgccatcgactccggaaaaacaaccagaagtaccggaatcaccaaaccagagaca
ccagacgcgccatcgactccaaaagatgagccgcaagttccatcaatcccagaggagcaa
ccaaaagagactccagcgccagaagaacctaaagaaggaagatactccgcaaacaccacaa
25 gcgccatcaactcctaaagaggaagcaccgaaggaagaagttccaacaccaccggctcca
tcagtaccagaagagcaacccaaaagaaactccaacaccagaagttccaaaacaagaagat
gttcaaccggaagcgccaaaatctgataaagtagaatctgacaaacaatgccagaaact
aagaaaccagatatgaagcaaccgaaggcagacgacatgcctaaggaacaaaagccgaaa
gccgacgagccaaaggcagagcaaccacaaatggacaaaccacaaatggaagctcctaag

aaagattcgggaagcgccaaaatctgataaagtagaaactgacaagcaattgccagaaact
aagcaaccagatatgaagcaaccgaaggcagacgacatgcctaaggaacaaaaaccgaaa
gccgacgagccaaaggcagagcaaccacaaatggacaaaccacaaatggaagctcctaag
aaagattcgggaagcaccaaaatctgataaagtagaaactgacaaaccaatgccagaaact
5 aaacaaccagatatgaagcaaccgaaggcagataaaccagaagcagagaaagctcaaatg
ccacggacagaggggtatgaagcctgaatctaaggcttcaatgatgccaaaagcagaggca
ccaaaagcaactttgccaaatacaggcgaagcaagtagcgcaataggctggctaggtggt
gctttagccactcttgccacaggcttgatctattcaaaaacaaaaagaagaatag

The amino acid sequence (SEQ ID NO: 9) coded by the above gene is:

10 MGKCI^NLYFKKEPIMSKQKV^SLLLLSTVVLGGLAFYSTTTVKAESLEPDVTSVNASDATNKPVVPNEEG
GDFLAE^EEELEDDDTLEELNEKAE^EVTEPSSPEALLQPRAMMSDSETSGMEEIPMNDEPSDNTTEEKVEKQ
QSPLIQ^TSNADYKSGKDQEKLR^TSVSINLLKAE^EGQIQWKVTFDTSEWSFN^VKHGGVYFILPNGLDLTKI
VDNNQH^DITASFPTDINDYRNSGQEKY^RFFSSKQGLDNENGFNSQWNWSAGQANPSETVNSW^KSGNRLSK
IYFINQ^ITDTELT^YTLTAKVTEPN^QSFPLLAVMKSFT^YTNSKSTEV^TSLGAREIT^LEKEKTLPPKENP
15 KPEPEAPKPDAPQAPSAPESPT^EEPPKEDAPAPSTPEKQPEVPE^SPNPETPDAPSTPKDEPQVPSIPEEQ
PKETPAPEEPKEDTPQTPQAPSTPKEEAPKEEV^TPPAPSVPEEQPKETPTPEVPKQEDVQPEAPKSDK
VESDKQMPETKKPDMKQPKADDMPKEQKPKADEPKAE^QPQMDKPQMEAPKKDSEAPKSDKVETDKQLPET
KQPDMKQPKADDMPKEQKPKADEPKAE^QPQMDKPQMEAPKKDSEAPKSDKVETDKPMPETKQPKDMKQPKA
DKPEAEKAQMPRTEGMKPE^SKASMPKAEAPKATL^PNTGEASSAIGWLGGALATLATGLYL^FKNKKEE

20 One fragment of the above coding gene was cloned. The nucleotide sequence (SEQ ID NO: 10) corresponding this fragment is:

GGGATCCGGGGTCAAGCTAATCCTAGTGAAACAGTAAACAGTTGGAAATCAGGCAATCGTTTGTCT
 AAGATTTATTTTCATAAATCAAATAACAGATACTACCGAATTGACCTATACTTTAACTGCTAAGGTA
 ACTGAACCAAATCAGCAATCATTCCCTCTTCTGGCAGTGATGAAGAGCTTTACGTATACAAACTCT
 AAGAGTACAGAGGTTACTTCGCTAGGTGCACGTGAGATTACCCTTGAGAAAGAAAAAACTCTACCA
 5 CCTAAGGAGAATCCAAAACCAGAGCCAGAAGCTCCAAAACCAGACGCTCCACAAGCGCCATCAGCA
 CCGGAATCTCCAACGGAAGAACCTAAGAAGGAAGACGCTCCAGCGCCATCGACTCCGGAAAAACAA
 CCAGAAGTACCGGAATCACCAAACCCAGAGACACCAGACGCGCCATCGACTCCAAAAGATGAGCCG
 CAAGTTCATCAATCCCAGAGGAGCAACCAAAGAGACTCCAGCGCCAGAAGAACCTAAGAAGGAA
 GATACTCCGCAAACACCACAAGCGCCATCAACTCCTAAAGAGGAAGCACCGAAGGAAGAAGTTCCA
 10 ACACCACCGGCTCCATCAGTACCAGAAGAGCAACCAAAGAAACTCCAACACCAGAAGTTCCAAA
 CAAGAAGATGTTCAACCGGAAGCGCCAAAATCTGATAAAGTAGAATCTGACAAACAAATGCCAGAA
 ACTAAGAAACCAGATATGAAGCAACCGAAGGCAGACGACATGCCTAAGGAACAAAAGCCGAAAGCC
 GACGAGCCAAAGGCAGAGCAACCACAAATGGACAAACCACAAATGGAAGCTCCTAAGAAAGATTCTG
 GAAGCGCCAAAATCTGATAAAGTAGAACTGACAAGCAATTGCCAGAAACTAAGCAACCAGATATG
 15 AAGCAACCGAAGGCAGACGACATGCCTAAGGAACAAAACCGAAAGCCGACGAGCCAAAGGCAGAG
 CAACCACAAATGGACAAACCACAAATGGAAGCTCCTAAGAAAGATTCTGGAAGCACCAAATCTGAT
 AAAGTAGAACTGACAAACCAATGCCAGAACTAAACAACCAGATATGAAGCAACCGAAGGCAGAT
 AAACCAGAAGCAGAGAAAGCTCAAATGCCACGGACAGAGGGTATGAAGCCTGAATCTAAGGCTTCA
 ATGATGCCAAAAGCAGAGGCACCAAAGCAACTTTGCCAAATACAGGCGAAGCAAGTAGCGCAATA
 20 GGCTGGCTAGGTGGTGCTTTAGCCACTCTTGCCACAGGCTTGTATCTACCCCGGG

The amino acid sequence coded by the above nucleotide sequence (SEQ ID NO: 11) is:

MKLKVTVNGTAYDVDVDVDKSHENPMGTILFGGGTGGAPAPAAGGAGAGKAGEGEIPAPLAGTVSK
 ILVKEGDTVKAGQTVLVLEAMKMETEINAPT DGKVEKVLVKERDAVQGGQLIKIGDLELIEGREA
 SAGIRGQANPSETVNSWKS GNRLSKIYFINQITDTELTYTLTAKVTEPNQQSFPLLAVMKSFTYT
 25 NSKSTEVTSLGAREITL EKEKTLPPKENPKPEPEAPKPDAPQAPSAPESPTEEPKKEDAPAPSTPE
 KQPEVPESP NPETPDAPSTPKDEPQVPSIPEEQPKETPAPEEPKKEDTPQTPQAPSTPKEEAPKEE
 VPTPPAPSVPEEQPKETPTPEVPKQEDVQPEAPKSDKVESDKQMPETKKPDMKQPKADDMPKEQKP
 KADEPKAEQPQMDKPQMEAPKKDSEAPKSDK VETDKQLPETKQPDMKQPKADDMPKEQKPKADEPK

AEQPQMDKFPQMEAPKKDSEAPKSDKVETDKPMPETKQPDMKQPKADKPEAEKAQMPRTEGMKPESK
ASMMPKAEAPKATLPNTGEASSAIGWLGGALATLATGLYLPRGGRDLVL

Theoretical pI/Mw: 4.86 / 62064.80.

Another object of the present invention are the proteins Ssu05_0473 and Ssu05_0272
5 or immunogenic derivatives thereof, in particular the fragment aa853-aa1252 of protein
Ssu05_0473, for use as medicament against Streptococcus suis colonization or
infection.

Said proteins and immunogenic derivatives can be used either singularly or in
combination with each other as medicament.

10 The present invention also refers to a vaccine at least one component selected from the
group consisting of protein Ssu05_0473, Ssu05_0272 and immunogenic derivatives
thereof, in particular the fragment aa853-aa1252 of protein Ssu05_0473, optionally
comprising an adjuvant. It is intended that the vaccine according to the present
invention can comprise as active ingredient the protein Ssu05_0473 or the protein
15 Ssu05_0272 or an immunogenic derivative thereof or any combination of said active
ingredients.

Another object of the present invention is a method for the diagnosis of bacterial
infections comprising the following steps:

- 20 a. Contacting an isolated biological sample of a subject with proteins
Ssu05_0473 and Ssu05_0272 and/or at least one immunogenic derivative
thereof, in particular the fragment aa853-aa1252 of protein Ssu05_0473;
- b. detecting antigen-antibody complex formation.

The present invention also refers to a method for the diagnosis of bacterial infections
comprising the following steps:

a. contacting an isolated biological sample of a subject with an antibody recognizing the protein Ssu05_0473 and Ssu05_0272 and/or at least one immunogenic derivative thereof, in particular the fragment aa853-aa1252 of protein Ssu05_0473;

5 b. detecting antigen-antibody complex formation.

Another object of the present invention is a kit for the diagnosis of Streptococcus suis infections containing the proteins Ssu05_0473 and Ssu05_0272 and/or at least one immunogenic derivatives thereof.

10 These and other objects of the present invention will now be illustrated in detail also by means of example.

Detailed description of the invention

The present invention provides the proteins Ssu05_0473 and Ssu05_0272 or their antigen fragments for use as medicament, in particular against Streptococcus suis colonization or infection.

15 According to the present invention, the same use is provided for immunogenic fragments of the protein Ssu05_0473, in particular the fragment aa853-aa1252.

20 Within the terms of the present invention, immunogenic fragment is an antigen fragment that substantially retains the capacity of eliciting an immune response, such as a protective response to a S. suis strain challenge when said fragment is administered to an animal.

In a preferred embodiment, the present invention provides a vaccine comprising the proteins Ssu05_0473 or Ssu05_0272 or both and/or at least one immunogenic fragment thereof, in particular the fragment aa853-aa1252 of protein Ssu05_0473.

In another preferred embodiment, the vaccine according to the present invention further comprises at least an adjuvant.

As used herein, the term "adjuvant" means a substance added to the composition of the invention to increase the composition's immunogenicity. The mechanism of how an adjuvant operates is not entirely known. Some adjuvants are believed to enhance the immune response (humoral and/or cellular response) by slowly releasing the antigen, while other adjuvants are strongly immunogenic in their own right and are believed to function synergistically. Known adjuvants include, but are not limited to, oil and water emulsions (for example, complete Freund's adjuvant and incomplete Freund's adjuvant), *Corynebacterium parvum*, Quil A, cytokines such as IL-12, Emulsigen-Plus(R), *Bacillus Calmette Guerin*, aluminum hydroxide, glucan, dextran sulfate, iron oxide, sodium alginate, Bacto Adjuvant, certain synthetic polymers such as poly amino acids and co-polymers of amino acids, saponin, paraffin oil, and muramyl dipeptide. Adjuvants also encompass genetic adjuvants such as immunomodulatory molecules encoded in a co-inoculated DNA, or as CpG oligonucleotides. The coinoculated DNA can be in the same plasmid construct as the plasmid immunogen or in a separate DNA vector.

Alternatively, the vaccine composition of the invention can comprise an antibody and/or a polynucleotide and/or an expression vector of the invention.

According to this further embodiment of the present invention, the antibody can be a purified polyclonal or monoclonal antibody that specifically binds to the *S. suis* proteins as defined above.

The antibodies used in the present invention may be prepared by a variety of methods known to one skilled in the art. For example, the protein of the invention may be administered to an animal in order to induce the production of polyclonal antibodies. Alternatively, antibodies used as described herein may be monoclonal antibodies, which are prepared using known hybridoma technologies (see, e.g., Hammerling et al, In

Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, NY, 1981 ; Charland, N., M. Jacques, S. Jacouture and M. Gottschalk. 1997. Characterization and protective activity of a monoclonal antibody against a capsular epitope shared by *Streptococcus suis* serotypes 1, 2 and Vz. *Microbiology* 143 (Pt 11): 3607-14).

5 With respect to antibodies used in the present invention, the term "specifically binds to" refers to antibodies that bind with a relatively high affinity to one or more epitopes of the proteins Ssu05_473 and Ssu05_0272 of the invention. As used herein, the term "relatively high affinity" means a binding affinity between the antibody and said protein of at least 10^6 M^{-1} , and preferably of at least about 10^7 M^{-1} and even more preferably
10 10^8 M^{-1} to 10^{10} M^{-1} . Determination of such affinity is preferably conducted under standard competitive binding immunoassay conditions which are common knowledge to one skilled in the art.

The present invention also concerns an isolated polynucleotide encoding the proteins of the invention for the same uses as the proteins itself. Preferably, the isolated
15 polynucleotide of the invention comprises a nucleotide sequence substantially identical to the sequence of Ssu05_0473 and Ssu05_0272 or their respective functional fragments thereof.

By "substantially identical" when referring to a nucleic acid sequence, it will be understood that the polynucleotide of the invention preferably has a nucleic acid
20 sequence which is at least 65% identical, more particularly 80% identical and even more particularly 95% identical to part or all of the encoding sequence known.

A "functional fragment", as is generally understood and used herein, refers to a nucleic acid sequence that encodes for a functional biological activity that is substantially similar to the biological activity of the whole nucleic acid sequence. In other words, and within
25 the context of the present invention, it preferably refers to a nucleic acid or fragment(s) thereof that substantially retains the capacity of encoding a polypeptide/protein which

elicits an immune response, and more preferably a protective response, to a *Streptococcus suis* strain challenge when administered to an animal.

In another embodiment, the invention is further directed to vector (e.g., cloning or expression vector) comprising a polynucleotide of the invention as defined above.

5 As used herein, the term "vector" refers to a polynucleotide construct designed for transduction/transfection of one or more cell types. Vectors may be, for example, "cloning vectors" which are designed for isolation, propagation and replication of inserted nucleotides, "expression vectors" which are designed for expression of a nucleotide sequence in a host cell, or a "viral vector" which is designed to result in the
10 production of a recombinant virus or virus-like particle, or "shuttle vectors", which comprises the attributes of more than one type of vectors.

A number of vectors suitable for stable transfection of cells and bacteria are available (e.g., plasmids, adenoviruses, baculoviruses, yeast baculoviruses, plant viruses, adeno-associated viruses, retroviruses, Herpes Simplex Viruses, Alphaviruses, Lentiviruses),
15 as are methods for constructing such cell lines. It will be understood that the present invention encompasses any type of vector comprising any of the polynucleotide molecule of the invention.

The proteins Ssu05_473 and Ssu05_0272, their fragments, polynucleotides encoding same and antibodies of the invention may be used in many ways in the treatment and/or
20 prevention of *Streptococcus suis*-associated diseases or infection caused by *S. suis*.

For instance, and according to an aspect of the invention, the proteins of the invention may be used as immunogens for the production of specific antibodies for the treatment and/or prevention of *Streptococcus suis* infection. Suitable antibodies may be determined using appropriate screening methods, for example by measuring the ability
25 of a particular antibody to passively protect against *Streptococcus suis* infection in a test model. Examples of an animal model are the mouse and pig models described in the examples herein.

According to another aspect, the polynucleotides encoding polypeptides of the invention or derivatives thereof may be used in a DNA immunization method.

That is, they can be incorporated into a vector which is replicable and expressible upon injection thereby producing the antigenic polypeptide in vivo. For example
5 polynucleotides may be incorporated into a plasmid vector under the control of the CMV promoter which is functional in eukaryotic cells. Preferably the vector is injected intramuscularly.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method or system such as direct injection of plasmid DNA
10 into muscles (Wolf et al. H M G (1992) 1 : 363, Turnes et al., Vaccine (1999), 17 : 2089, Le et al. , Vaccine (2000) 18 : 1893, Alves et al. , Vaccine (2001)19 : 788), injection of plasmid DNA with or without adjuvants (Ulmer et al. , Vaccine (1999) 18: 18, MacLaughlin et al. , J. Control Release (1998) 56: 259, Hartikka et al. , Gene Ther. (2000) 7: 1171-82, Benvenisty and Reshef, PNAS USA (1986) 83: 9551 , Singh et al. ,
15 PNAS USA (2000) 97: 811), targeting cells by delivery of DNA complexed with specific carriers (Wa et al. , J Biol Chem (1989) 264: 16985, Chaplin et al., Infect. Immun. (1999) 67:6434), injection of plasmid complexed or encapsulated in various forms of liposomes (Ishii et al. , AIDS Research and Human Retroviruses (1997) 13: 142, Perrie et al., Vaccine (2001) 19:3301), administration of DNA with different methods of
20 bombardment (Tang et al., Nature (1992) 356: 152, Eisenbraun et al. , DNA Cell Biol (1993) 12: 791 , Chen et al. , Vaccine (2001) 19:2908), and administration of DNA with lived vectors (Tubulekas et al. , Gene (1997) 190: 191 , Pushko et al., Virology (1997) 239: 389, Spreng et al. FEMS (2000) 27: 299, Dietrich et al., Vaccine (2001) 19: 2506).

A further aspect of the invention is the use of the antibodies directed to the polypeptides
25 of the invention for passive immunization. One could use the antibodies described in the present application.

Therefore, according to a general aspect of the invention, the term “immunogenic derivative” comprises the above explained immunogenic fragments of the protein of the present invention, the antibodies, polynucleotides and vectors.

Yet, a further embodiment of the present invention is to provide a method for treating
5 and/or preventing a Streptococcus suis-associated disease or infection in an animal. The method of the invention comprises the step of administering to the animal a composition according to the invention.

Further agents can be added to the composition of the invention. For instance, the composition of the invention may also comprise agents such as drugs,
10 immunostimulants (such as [alpha]-interferon, [beta]-interferon, [gamma]-interferon, granulocyte macrophage colony stimulator factor (GM-CSF), macrophage colony stimulator factor (M-CSF), and interleukin 2 (IL-2)), antioxidants, surfactants, flavoring agents, volatile oils, buffering agents, dispersants, propellants, and preservatives. For preparing such compositions, methods well known in the art may be used. The amount
15 of the components or the elements of the composition of the invention is preferably a therapeutically effective amount. A therapeutically effective amount of the contemplated component is the amount necessary to allow the same to perform their immunological role without causing overly negative effects in the host to which the composition is administered. The exact amount of the components to be used and the composition to
20 be administered will vary according to factors such as the type of condition being treated, the type and age of the animal to be treated, the mode of administration, as well as the other ingredients in the composition.

The composition of the invention may be given to an animal through various routes of administration. For instance, the composition may be administered in the form of sterile
25 injectable preparations, such as sterile injectable aqueous or oleaginous suspensions. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparations may also be sterile injectable solutions or suspensions in non-toxic

parenterally-acceptable diluents or solvents. They may be given parenterally, for example intravenously, intramuscularly or subcutaneously by injection, by infusion or per os. Suitable dosages will vary, depending upon factors such as the amount of each of the components in the composition, the desired effect (short or long term), the route
5 of administration, the age and the weight of the animal to be treated. Any other methods well known in the art may be used for administering the composition of the invention.

The proteins Ssu05_0473 and Ssu05_0272, polynucleotides encoding same and antibodies of the invention may also be used in different ways in the detection and diagnosis of Streptococcus suis-associated diseases or infections caused by S. suis.

10 In this connection and in a further embodiment, the present invention provides a method for detecting the presence or absence of a Streptococcus suis strain in a sample, comprising the steps of:

a) contacting the sample with an antibody of the invention as defined above for a time and under conditions sufficient to form a complex; and

15 b) detecting the presence or absence of the complex formed in a).

As used herein, the term "sample" refers to a variety of sample types obtained from an animal and can be used in a diagnostic or detection assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue culture or cells derived therefrom.

20 Yet, in another embodiment, the present invention provides a method for detecting the presence or absence of antibodies raised against a Streptococcus suis strain in a sample, comprising the steps of:

a) contacting the sample with a protein and/or a polypeptide and or an immunogenic derivative of the invention as defined above for a time and under
25 conditions sufficient to form an immune complex; and

b) detecting the presence or absence of the immune complex formed in a).

One skilled in the art will recognize that this diagnostic test may take several forms, including an immunological test such as an enzyme-linked immunosorbent assay (ELISA) or a radioimmunoassay, essentially to determine whether antibodies specific for
5 the protein are present in an organism.

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain an antibody or
10 fragment thereof that specifically binds to proteins Ssu05_0473 and Ssu05_0272 of the invention. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay.

In this connection, the present invention also provides a diagnostic kit for the detection of the presence or absence of antibodies indicative of Streptococcus suis strain,
15 comprising:

- a protein Ssu05_0473 and/or a protein Ssu05_0272 and/or an immunogenic derivative thereof according to the invention;
- a reagent to detect polypeptide-antibody immune complex;
- a biological reference sample lacking antibodies that immunologically bind with said
20 polypeptide; and
- a comparison sample comprising antibodies which can specifically bind to said polypeptide; wherein said polypeptide, reagent, biological reference sample, and comparison sample are present in an amount sufficient to perform said detection.

Another diagnostic kit preferably contemplated is a kit for the detection of the presence
25 or absence of polypeptides indicative of Streptococcus suis strain, comprising:

- an antibody according to the invention;
 - a reagent to detect polypeptide-antibody immune complex;
 - a biological reference sample polypeptides that immunologically binds with said antibody; and
- 5 - a comparison sample comprising polypeptides which can specifically bind to said peptide; wherein said antibody, reagent, biological reference sample, and comparison sample are present in an amount sufficient to perform said detection.

The following example further illustrates the invention.

Example

10 Material and Methods:

Bacterial strains and growth.

Streptococcus suis serotype 2, strain 235/02, isolated from an infected pig in Córdoba, Spain in 2002, was grown in Todd-Hewitt broth supplemented with 0.5% yeast extract (THY) at 37 °C and 5% CO₂, until an OD₆₀₀ of 0.25 (mid-exponential phase) was
15 reached.

Surface digestion of live cells and viability assays.

One hundred ml of bacteria from mid-exponential growth phase (corresponding to approximately 10¹⁰ cells at OD₆₀₀ = 0.25) were harvested by centrifugation at 3,500 × g for 10 min at 4 °C, and washed three times with PBS. Cells were resuspended in 0.8 ml
20 of incubation buffer consisting of PBS/30% sucrose (pH 7.4 for trypsin digestion and pH 6.0 for proteinase K digestion). Proteolytic reactions were carried out with trypsin (Promega) at 10 µg/ml or proteinase K (Sigma) at 5 µg/ml, for 20 min at 37 °C. Controls were carried out without adding any enzyme. The digestion mixtures were centrifuged at 3,500 × g for 10 min at 4 °C, and the supernatants (containing the peptides and large

poplypeptides not fully digested) were filtered using 0.22- μ m pore-size filters (Millipore). An aliquot of each digestion reaction was re-digested each one with the same enzyme and concentration, trypsin digestion for 2 h and proteinase-K digestion for 20 min. Protease reactions were stopped with formic acid at 0.1% final concentration. Before
5 analysis, salts were removed by using commercial mini-cartridges HLB-Oasis (Waters) and then eluting the peptides with increasing concentrations of acetonitrile, according to manufacturer's instructions. Peptide fractions were concentrated with a vacuum concentrator (Eppendorf), and kept in low-binding tubes at -20 °C until further analysis. Viability of treated and non-treated bacteria with proteases was assayed by counting
10 CFUs (colony-forming units) in THY plates containing 5% defibrinated sheep blood.

LC/MS/MS analysis.

All analyses were performed with a Surveyor HPLC System in tandem with a Finnigan LTQ mass spectrometer (Thermo Fisher Scientific, San Jose, USA) equipped with nanoelectrospray ionization interface (nESI). The separation column was 150 mm x
15 0.150 mm ProteoPep2 C18 (New Objective, USA) at a postsplit flow rate of 1 μ l/min. For trapping of the digest a 5 mm x 0.3 mm precolumn Zorbax 300 SB-C18 (Agilent Technologies, Germany) was used. One fourth of the total sample volume, corresponding to 5 μ l, was trapped at a flow rate of 10 μ l/min for 10 minutes and 5% acetonitrile/0.1% formic acid. After that, the trapping column was switched on-line with
20 the separation column and the gradient was started. Peptides were eluted with a 60-min gradient of 5–40% of acetonitrile/0.1% formic acid solution at a 250 nl/min flow rate. All separations were performed using a gradient of 5-40% solvent B for 60 minutes. MS data (Full Scan) were acquired in the positive ion mode over the 400-1500 m/z range. MS/MS data were acquired in dependent scan mode, selecting automatically the five
25 most intense ions for fragmentation, with dynamic exclusion set to on. In all cases, a nESI spray voltage of 1.9 kV was used.

Database searching and protein identification.

Search and identification of peptides were performed using in batch mode the raw MS/MS data with a licensed version of MASCOT, in a non-redundant local database containing the 2,185 proteins derived from the complete genome sequence of Streptococcus suis strain 05ZYH33 (RefSeq NC_009442 downloaded from
5 ftp://ftp.ncbi.nih.gov/genomes/Bacteria/Streptococcus_suis_05ZYH33, NCBI nr version 20071013). The MASCOT search parameters were: (i) species, Streptococcus suis strain 05ZYH33; (ii) allowed number of missed cleavages (only for trypsin digestion), 4; (iii) variable post-translational modifications, methionine oxidation, and deamidation of asparagine and glutamine residues; (iv) peptide mass tolerance, ± 500 p.p.m.; (v)
10 fragment mass tolerance, ± 0.6 Da and (vi) peptide charge, from +1 to +4. The score thresholds for acceptance of protein identifications from at least one peptide were set by MASCOT as 19 for trypsin cleavage and 34 for proteinase K digestion. All spectra corresponding to positive identifications or near the thresholds were manually inspected.

15 Bioinformatic analysis of protein sequences.

Computational predictions of subcellular localization were carried out using the web-based algorithm PSORTb v 2.0 (<http://www.psort.org/psortb>). Feature-based algorithms were also used to contrast PSORTb predictions, especially when it returned an “unknown” output: TMHMM 2.0 (<http://www.cbs.dtu.dk/services/TMHMM-2.0>) for
20 searching transmembrane helices; SignalP 3.0 (<http://www.cbs.dtu.dk/services/SignalP>) for type-I signal peptides: those proteins containing only a cleavable type-I signal peptide as featured sequence were classed as secreted; LipoP (<http://www.cbs.dtu.dk/services/LipoP>) for identifying type-II signal peptides, which are characteristic of lipoproteins. Similarity searches were carried out using the BLAST suite
25 (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). Gene Ontology (GO) annotations were retrieved using the AmiGO browser (<http://amigo.geneontology.org/cgi-bin/amigo/go.cgi>). Additional information on protein families, motifs, predictions of

subcellular localization and status of the protein was retrieved from UniProt Knowledgebase (<http://www.uniprot.org>).

Expression and purification of *Streptococcus suis* proteins and preparation of immune sera

5 Cloning, expression and purification of recombinant proteins, and preparation of immune sera, were performed as follows. Briefly, the chromosomal DNA of strain 235/02 was used for gene amplification. PCR primers were designed so as to amplify genes or gene fragments without predicted signal peptide coding sequences. The sequences of the primers used for amplifying the fragment FA1 of Ssu05_0473 are:

10 FA1_Up CGCGGATCCGGGAGTCTGCTAGC (SEQ ID NO: 12) with restriction site (BamHI),

FA1_Low GAGTCATTCTGCGGCCGCCAAAGGTTTTCCG (SEQ ID NO: 13) with restriction site (NotI).

The sequences of the primers used for amplifying the fragment FA3 of Ssu05_0473 are:

15 FA3_up CGCGGATCCGGAATAAGATGCCTG (SEQ ID NO: 14) with BamHI restriction site,

FA3_low GAGTCATTCTGCGGCCGCCGGTTATGTGATAG (SEQ ID NO: 15) with NotI restriction site.

The sequences of the primers used for amplifying the fragment of Ssu05_0272 are:

20 up CGCGGATCCGGGGTCAAGCTAATC (SEQ ID NO: 16) with restriction site (BamHI),

Low_Sma TCCGGACCCGGGGTAGATAACAAGCC (SEQ ID NO: 17) with restriction site (SmaI).

PCR products were introduced into a commercial vector [PinPoint Xa1] so as to generate biotin-tagged recombinant proteins (PinPoint Xa1 Protein Purification System, Promega). E.coli JM109 cells were used as the recipient. Recombinant proteins were affinity-purified with SoftLink Soft Release Avidin Resin (Promega), and 3 doses of 50 µg each were used to immunize groups of adult CD1 mice (Charles River, 10 mice per group) at days 0, 21 and 42. Mice were bled 2 weeks after the third immunization. The sera of each group were pooled and stored at -80°C.

FACS analysis of surface proteins

Bacteria were grown in THB until mid-exponential phase was reached, then washed twice with PBS, fixed in 2% paraformaldehyde, suspended in NCS (Newborn Calf Serum, Sigma), incubated for 20 min at RT and dispensed in a 96-well plate (20µl/well). Eighty µl of pre-immune or immune mouse sera, diluted in PBS containing 0.1% BSA, were added to the bacterial suspension to a final dilution of 1:80 and incubation was performed on ice for 30 min. After washing twice with 0.1%BSA in PBS, bacteria were incubated on ice for 30 min in 10 µl of Goat Anti-Mouse IgG, F(ab')₂ fragment-specific-R-Phycoerythrin-conjugated diluted 1:50 in PBS containing 0.1% BSA-20% NCS. Following incubation, bacteria were washed with PBS-0.1% BSA, suspended in 200µl PBS and analyzed using a FACS Calibur cytometer (Becton Dickinson, Mountain View, CA, USA) and Cell Quest Software (Becton Dickinson).

20 Screening of protective antigens in the mouse model

Eight-week-old, specific pathogen-free CD1 mice (Charles River, 10 mice/group) were immunized with 50 µg of recombinant proteins administered intraperitoneally at 0, 21 and 42 days with complete Freund adjuvant (CFA) (priming dose) and incomplete Freund adjuvant (IFA) (booster doses). Positive controls consisted of immunization with Sao-like protein. A negative control group consisted of animals immunized with a biotin-tagged irrelevant protein (chloramphenicol acetyl transferase, CAT-BIO). Blood samples were collected before the first (pre-immune sera) and after the third immunizations

(immune sera). Immunized mice were challenged intravenously (i.v.) with 100 µl of THB containing 2×10^7 colony forming units (CFUs) of *S. suis* strain 235/02 grown in THB broth at mid-exponential phase. Protection was determined from the bacterial burden in the kidneys at 4 days after intravenous infection, by counting the CFUs after plating in TH plates supplemented with 0.5% yeast extract and 5% defibrinated sheep blood.

Results:

Table 1 shows the list of identified proteins by the previously described proteomics approach when applied to *Streptococcus suis* strain 235/02, serotype 2:

Table 1

10 Surface proteins identified by LC/MS/MS after protease treatment of cultured live cells of *Streptococcus suis* serotype 2, strain 235/02. The table reports: 1) the accession number of the genes encoding the identified proteins, 2) the predicted cellular localization of the proteins, 3) the algorithms used for such predictions.

| Gene locus, Annotated protein function | Predicted subcellular localization | Prediction algorithm |
|---|---------------------------------------|----------------------|
| ssu05_0753, MRP | Cell wall | PSORTb v 2.0 |
| ssu05_1982, Subtilisin-like serine protease | Cell wall | PSORTb v 2.0 |
| ssu05_1968, DNA nuclease | Cell wall | PSORTb v 2.0 |
| ssu05_0196, hypothetical protein SSU05_0196 | Cell wall | PSORTb v 2.0 |
| ssu05_1311, hypothetical protein SSU05_1311 | Cell wall | PSORTb v 2.0 |

| | | |
|---|-------------|--------------|
| ssu05_0965, agglutinin receptor | Cell wall | PSORTb v 2.0 |
| ssu05_2064, Type II secretory pathway, pullulanase PulA and related glycosidases | Cell wall | PSORTb v 2.0 |
| ssu05_1371, Ribonucleases G and E | Cell wall | PSORTb v 2.0 |
| ssu05_1295, hypothetical protein SSU05_1295 | Cell wall | PSORTb v 2.0 |
| ssu05_0214, ABC-type xylose transport system, periplasmic component | Cell wall | PSORTb v 2.0 |
| ssu05_1663, Methyl-accepting chemotaxis protein | Cell wall | PSORTb v 2.0 |
| ssu05_0473, Ribonucleases G and E | Cell wall | PSORTb v 2.0 |
| Ssu05_0272, Translation initiation factor 2 (IF-2; GTPase) | Cell wall | PSORTb v 2.0 |
| ssu05_0700, ATPase (PilT family) | Lipoprotein | LipoP |
| ssu05_1083, Uncharacterized ABC-type transport system, periplasmic component/surface lipoprotein | Lipoprotein | LipoP |

| | | |
|---|-------------|---------------------|
| ssu05_2133, ABC transporter substrate- binding protein - maltose/maltodextrin | Lipoprotein | LipoP |
| ssu05_0513, Membrane-fusion protein | Membrane | TMHMM |
| ssu05_1022, hypothetical protein SSU05_1022 | Membrane | TMHMM |
| ssu05_1635, Predicted xylanase/chitin deacetylase | Membrane | TMHMM |
| ssu05_1354, Cell division protein FtsI/penicillin-binding protein 2 | Membrane | TMHMM |
| ssu05_1509, Negative regulator of septation ring formation | Membrane | TMHMM |
| ssu05_2173, LysM repeat protein | Membrane | TMHMM |
| ssu05_1579, Ammonia permease | Membrane | PSORTb v 2.0, TMHMM |
| ssu05_1292, Phosphoglycerol transferase and related proteins, alkaline phosphatase superfamily | Membrane | PSORTb v 2.0, TMHMM |
| ssu05_1380, ABC-type antimicrobial peptide transport system, permease | Membrane | TMHMM |

| | | |
|---|----------|---------------------|
| component | | |
| ssu05_1282, Predicted membrane protein | Membrane | PSORTb v 2.0, TMHMM |
| ssu05_0332, hypothetical protein SSU05_0332 | Secreted | SignalP |
| ssu05_0811, Subtilisin-like serine protease | Secreted | SignalP |
| ssu05_1682, extracellular serine protease | Secreted | SignalP |

For three identified proteins, coded by the loci ssu05_1371, ssu05_0473 (both annotated as “ribonucleases G and E”) and ssu05_0272 (annotated as “Translation initiation factor 2 (IF-2; GTPase)), the assigned functions were not in agreement with their primary sequences. When examining the sequences at the amino acid level, they

5 showed the canonical architecture of the cell-wall attached proteins.

Ribonucleases G and E are a family of endonucleases involved in RNA processing: their cellular localization is, therefore, cytoplasmic. They are mainly present in Gram-negative bacteria, and rarely detected in Gram-positive organisms. In fact, Ssu05_1371 is not similar to any of the proteins annotated as ribonucleases G and E in the

10 databases. However, similarity (83% identity) was found to protein Sao from *Streptococcus suis* (GenBank accession number AY864331, and named “surface protein SP1” in the not yet fully annotated strain 89/1591), which has been shown to be surface-located and also to protect immunised animals against infection. Moreover, Ssu05_1371 was highly similar to other streptococcal proteins that have characteristic

15 functions attributed to surface proteins, as binding to the extracellular matrix (ECM) of the host cells. These proteins are known generally as adhesins. Adhesins mediate adherence to host cells or tissues during the first steps of infection.

The locus *ssu05_0473* codes for a large protein of 1603 amino acids, showing the structure of a cell-wall protein with the LPXTG (SEQ ID NO: 1) motif. This locus is in a region that is analogous to the pilus island 2b (PI-2b) from *Streptococcus agalactiae* COH1, which has recently been found in the draft genome of *S. suis* P1/7 (Fittipaldi et al, Appl Environ Microbiol 2007). Pili are filamentous structures that, in Gram-positive organisms, serve to adhere and invade host cells. Protein *Ssu05_0473* was not identified by trypsin digestion and this is reminiscent of the *S. pyogenes* pilin proteins which were previously named “T antigens” (for “trypsin resistant”). The identification of protein *Ssu05_0473* was achieved by two peptides after proteinase K treatment.

10 Protein *Ssu05_0272* has been also wrongly annotated as “Translation initiation factor 2 (IF-2; GTP-ase)” (translation initiation factors are cytoplasmic proteins), 698 amino acids long and also containing the cell-wall sorting signal. No strong similarity was found to other proteins. However, slight similarity was found to proteins from other Gram-positive organisms with adhesin function, or even to a collagen-like protein from

15 *Streptococcus equis* subsp. *zooepidemicus* (locus tag: *sez_0729*) or a collagen-like surface protein from *Streptococcus pyogenes*. Pattern and profile searches (InterPro, Pfam, PROSITE) did not reveal other domains apart from Gram-positive, LPXTG-dependent (SEQ ID NO: 1) anchoring to the cell wall. These three proteins were used in a protection assay in which animals were immunised intraperitoneally with said proteins

20 (boosts at days 0, 14 and 28), and then challenged intravenously (at day 49) with *S. suis* strain 235/02. Protective capacity was measured by means of the decrease in CFUs recovered from the kidneys of infected animals: the lower the number of CFUs, the more the protein protected. Protein *Ssu05_1371*, previously identified as Sao and demonstrated to protect immunised mice (Li et al, 2006), was used as positive control.

25 Two recombinant fragments of protein *Ssu05_0473* (aa51-aa451 and aa853-aa1252) were tested. Table 2 shows the mean values of the CFUs/ml from kidneys after 96-hours challenge. Fragment aa853-1252 of *Ssu05_0473* and protein *Ssu05_1371* showed a clear protective activity with respect to the control (CAT-bio), and the protection capacity was especially high for animals immunised with *Ssu05_0272*.

Table 2

| Immunogen | Kidney CFU/ml (96 h after challenge) |
|---|--|
| K- (CAT-bio): Chloramphenicol acetyltransferase –biotine | 9×10^4 (DS= 1×10^5) |
| SSU_0473 /FA1 (400aa;a51-a451) | 1×10^5 (DS= $1,9 \times 10^5$) |
| SSU_0473/FA3 (399 aa;a853-a1252) | 1×10^4 (DS= 3×10^4) |
| SSU_1371 (551aa;a50-a601) | 3×10^4 (DS= 1×10^5) |
| SSU_0272 | 5×10^3 (DS= 6×10^3) |

Table 3 shows the percentage of protected animals for each immunogen (ten animals were used per group). The higher percentages were obtained for the fragment aa853-aa1252 of Ssu05_0473 and for protein Ssu05_1371.

5

Table 3

| Immunogen | Number of protected mice/total(%) |
|---|-----------------------------------|
| K- (CAT-bio): Chloramphenicol acetyltransferase –biotine | 1/10 (10%) |
| SSU_0473 /FA1 (400aa;a51-a451) | 2/10 (20%) |
| SSU_0473/FA3 (399 aa;a853-a1252) | 7/10 (70%) |
| SSU_1371 (551aa;a50-a601) | 8/10 (80%) |
| SSU_0272 | 3/10 (30%) |

Industrial Application

The present invention relates to the medical field, in particular to the diagnosis and cure of infection of *Streptococcus suis*.

The present invention provides a vaccine against *Streptococcus suis*, useful to prevent and cure the infection caused by said pathogen, and a kit for the detection of *Streptococcus suis* infection with high reliability which can be fast and disposable.

CLAIMS

1. Protein Ssu05_0473 and Ssu05_0272 and immunogenic derivatives thereof for use as a medicament.
- 5 2. The immunogenic derivative of the protein Ssu05_0473 of claim 1, which is the fragment aa853-aa1252 for use as a medicament.
3. Protein Ssu05_0473 and Ssu05_0272 and immunogenic fragments thereof for use as medicament against Streptococcus suis colonization or infection.
4. The immunogenic derivative of the protein Ssu05_0473 of claim 3, which is the
10 fragment aa853-aa1252 for use as a medicament against Streptococcus suis colonization or infection.
5. A vaccine comprising at least one component selected from the group consisting of protein Ssu05_0473, Ssu05_0272 and immunogenic derivatives thereof.
6. The vaccine of claim 5 wherein said immunogenic derivative is the fragment
15 aa853-aa1252 of the protein Ssu05_0473.
7. The vaccine according to claim 5 or 6, further comprising at least an adjuvant.
8. Method for the diagnosis of bacterial infections comprising the following steps:
 - a. contacting an isolated biological sample of a subject with the protein of claim 1 and/or at least one immunogenic derivative thereof;
 - 20 b. detecting antigen-antibody complex formation.
9. Method according to claim 8, wherein said derivative is the fragment aa853-aa1252 of the protein Ssu05_0473.
10. Method for the diagnosis of bacterial infections comprising the following steps:

- a. contacting an isolated biological sample of a subject with an antibody recognizing the protein of claim 1 and/or at least one immunogenic derivative thereof;
 - b. detecting antigen-antibody complex formation.
- 5 11. Method according to claim 10, wherein said derivative is the fragment aa853-aa1252 of the protein Ssu05_0473.
12. Kit for the diagnosis of Streptococcus suis infections containing the protein and/or at least one immunogenic derivatives thereof of Claim 1.
- 10 13. Kit for the diagnosis of Streptococcus suis infections containing an antibody recognizing the protein and/or at least one immunogenic derivatives thereof of Claim 1.