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(54) Title: INTERACTION OF MORAXELLA CATARRHALIS WITH EPITHELIAL CELLS, EXTRACELLULAR MATRIX PROTEINS AND THE COMPLEMENT SYSTEM

(57) Abstract: The present invention relates to extracellular matrix proteins of Moraxella catarrhalis and their ability to interact with epithelial cells via cellassociated fibronectin and laminin, and also to their ability to inhibit the complement system. These extracellular proteins are useful in the preparation of vaccines. The present invention provides peptides interacting with the fibronectin, laminin and complement system.

INTERACTION OF MORAXELLA CATARRHALIS WITH EPITHELIAL CELLS, EXTRACELLULAR MATRIX PROTEINS AND THE COMPLEMENT SYSTEM

Technical field of the invention

The present invention relates to *Moraxella catarrhalis* and their ability to interact with epithelial cells via extracellular matrix proteins such as fibronectin and laminin, and also to their ability to inhibit the complement system. The interaction with these extracellular proteins is useful in the preparation of vaccines.

Background art

The ability to bind epithelial cells is of great importance for several bacterial species. For example, *Staphylococcus aureus* and *Streptococcus pyogenes* possess fibronectin binding proteins (FnBP) with related sequence organization. These FnBP are known as Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs). They exploit the modular structure of fibronectin forming extended tandem beta-zippers in its binding to fibronectin.[27, 39, 47, 73] The function is to mediate bacterial adhesion and invasion of host cells.

The important mucosal pathogen *Moraxella catarrhalis* is the third leading bacterial cause of acute otitis media in children after *Streptococcus pneumoniae* and *Haemophilus influenzae.*[14, 40, 55] *M. catarrhalis* is also one of the most common inhabitants of the pharynx of healthy children.

Furthermore, *M. catarrhalis* is also a common cause of sinusitis and lower respiratory tract infections in adults with chronic obstructive pulmonary disease (COPD). [74] The success of this species in patients with COPD is probably related in part to its large repertoire of adhesins.

Recent years focus of research has been on the outer membrane proteins and their interactions with the human host.[6, 48, 56] Some of these outer membrane proteins appear to have adhesive functions including amongst others, *M. catarrhalis* IgD binding protein (MID, also designated Hag), protein CD, *M. catarrhalis* adherence protein (McaP) and the ubiquitous surface proteins (Usp).[1, 22, 33, 48, 61, 81, 84]

Summary of the invention

In view of the fact that *M. catarrhalis* has been found to be such a leading cause of infections in the upper and lower airways, there is a current need to develop vaccines which can be used against *M. catarrhalis*.

The aim of the present invention has therefore been to find out in which way *M. catarrhalis* interacts with epithelial cells in the body and affects the immune system. In this way, substances that can act as vaccines against *M. catarrhalis* can be developed.

In this study, using *M. catarrhalis* mutants derived from clinical isolates, the inventors have been able to show that both UspA1 and A2 bind fibronectin and laminin. Furthermore, the inventors have been able to show that *M. catarrhalis* interfere with the classical pathway of the complement system, and also to elucidate in which way they interfere.

Many bacteria adhere to epithelial cells via fibronectin binding MSCRAMMS.[54, 77] *Pseudomonas aeruginosa* has a FnBP that binds to cellular associated fibronectin on nasal epithelial cells.[69] Blocking the bacteria-fibronectin protein interactions may help the host tissue to overcome the infection. In fact, it has been shown that antibodies against a *S. aureus* FnBP resulted in rapid clearance of the bacteria in infected mice.[71]

Recombinant truncated UspA1/A2 proteins together with smaller fragments spanning the entire molecule have been

tested according to the present invention for fibronectin binding. Both UspA1 and A2 bound fibronectin and the fibronectin binding domains were found to be located within $UspA1^{299-452}$ and $UspA2^{165-318}$. These two truncated proteins both inhibited binding of *M. catarrhalis* to Chang conjunctival epithelial cells to a similar extent as anti-fibronectin antibodies. The observations made show that both *M. catarrhalis* UspA1 and A2 are involved in the adherence to epithelial cells via cell-associated fibronectin. The biologically active sites within $UspA1^{299-452}$ and $UspA2^{165-318}$ are therefore suggested as potential candidates to be included in a vaccine against *M. catarrhalis*.

Further, the inventors have studied and characterized binding of *M. catarrhalis* to laminin. *M. catarrhalis* is a common cause of infectious exacerbations in patients with COPD. The success of this species in patients with COPD is probably related in part to its large repertoire of adhesins. In addition, there are pathological changes such as loss of epithelial integrity with exposure of basement membrane where the laminin layer itself is thickened in smokers.[4] Some pathogens have been shown to be able to bind laminin and this may contribute to their ability to adhere to such damaged and denuded mucosal surfaces. These include pathogens known to cause significant disease in the airways such as *S. aureus* and *P. aeruginosa* amongst others.[7, 63] The present inventors have been able to show that *M. catarrhalis* ubiquitous surface protein (Usp) A1 and A2 also bind to laminin. Laminin binding domains of UspA1 and A2 were, amongst others, found within the N-terminal halves of $UspA1^{50-491}$ and $UspA2^{30-351}$. These domains are also containing the fibronectin binding domains. However, the smallest fragments that bound fibronectin, $UspA1^{299-452}$ and $UspA2^{165-318}$, did not bind laminin to any appreciable extent. Fragments smaller than the N-terminal half of UspA1 ($UspA1^{50-491}$) lose all its laminin binding ability, whereas with

UspA2, only UspA2$^{30-170}$ bound laminin albeit at a lower level than the whole recombinant protein (UspA2$^{30-539}$). These findings suggest that different parts of the molecule might have different functional roles. UspA1$^{50-770}$ was also found to have laminin binding properties.

Comparing the smallest laminin binding regions of UspA1 and A2, we find that there is, however, little similarity by way of amino acid homology between UspA2$^{30-170}$ and UspA1$^{50-491}$ (data not shown). This is not surprising as it is a known fact that both proteins have a 'lollipop'-shaped globular head structure despite having only 22% identity in both N terminal halves.[2, 32]

The biologically active sites within UspA1$^{50-770}$ and UspA2$^{30-539}$ are suggested as potential candidates to be included in a vaccine against *M. catarrhalis*.

Finally, the inventors have studied the interaction between *M. catarrhalis* ubiquitous surface proteins A1 and A2 and the innate immune system, and have found that *M. catarrhalis* interferes with the complement system. The complement system is one of the first lines of innate defence against pathogenic microorganisms, and activation of this system leads to a cascade of protein deposition on the bacterial surface resulting in formation of the membrane attack complex or opsonization of the pathogen followed by phagocytosis. [85, 86] One of the most important complement proteins is C3, which is present in the circulation in a concentration similar to some immunoglobulins (1-1.2 mg/ml). C3 does not only play a crucial role as an opsonin, but also is the common link between the classical, lectin and alternative pathways of the complement activation. The alternative pathway functions as amplification loop for the classical and lectin pathways and can also be spontaneously activated by covalent attachment of C3 to the surface of a microbe in the absence of complement inhibitors. C3 deposition requires the presence of an internal thioester

bond, formed in the native protein by the proximity of a sulfhydryl group (Cys^{1010}) and a glutamyl carbonyl (Gln^{1012}) on the C3 α-chain.[76] Proteolytic cleavage of a 77-residue peptide from the amino terminus of the C3 α-chain generates C3a (anaphylatoxin) and C3b. Attachment of C3b is then accomplished through a covalent link between the carbonyl group of the metastable thioester and either $-NH_2$ or -OH groups of proteins or carbohydrate structures on the activator surface. [36, 37] *M. catarrhalis* UspA1 and A2 have been found to non-covalently and in a dose dependent manner bind both the third component of complement (C3) from EDTA-treated serum and methylamine treated C3 (C3met). $UspA1^{50-770}$ and $UspA2^{30-539}$ have been found to bind to C3 and C3met. The C3-binding region for UspA2 was found to mainly be localised in $UspA2^{200-458}$. UspA1 has however been found to have a minor role in the interactions. The biologically active sites within $UspA1^{50-770}$ and $UspA2^{30-539}$ are suggested as potential candidates to be included in a vaccine against *M. catarrhalis*.

The UspA family consists of UspA1 (molecular weight 88 kDa), UspA2 (62 kDa), and the hybrid protein UspA2H (92 kDa).[2, 43] These proteins migrate as high molecular mass complexes in SDS-PAGE, are relatively conserved and hence important vaccine candidates. The amino acid sequences of UspA1 and A2 are 43 % identical and have 140 amino acid residues that are 93 % identical. [2] In a series of 108 *M. catarrhalis* nasopharyngeal isolates from young children with otitis media, *uspA1* and *uspA2* genes were detected in 107 (99 %) and 108 (100 %) of the isolates, respectively. Twenty-one percent were identified as having the hybrid variant gene *uspA2H*.[50] Moreover, it is known that naturally acquired antibodies to UspA1 and A2 are bactericidal.[15]

Several functions have been attributed to the UspA family of proteins. UspA1 expression is essential for the attachment of *M. catarrhalis* to Chang conjunctival epithe-

lial cells and Hep-2 laryngeal epithelial cells.[43, 49] In a more recent study, UspA1 was shown to bind carcinoembryonic antigen related cell adhesion molecules (CEACAM) expressed in the lung epithelial cell line A549.[31] Purified UspA1 has also been shown to bind fibronectin in dot blot experiments while purified UspA2 did not.[49] Both UspA1 and A2 may play important roles for *M. catarrhalis* serum resistance.[1, 5, 58, 60]

The present invention demonstrates that both UspA1 and A2 are determinants for *M. catarrhalis* binding to fibronectin and laminin in the clinical isolates *M. catarrhalis* BBH18 and RH4. Interestingly, recombinant UspA1 and A2 derived from *M. catarrhalis* Bc5 both bound fibronectin to the same extent. The binding domains for fibronectin were found within amino acid residues 299 to 452 of UspA1 and 165 to 318 of UspA2. These two domains share 31 amino acid residues sequence identity. Importantly, truncated protein fragments containing these residues in UspA1 and UspA2 were able to inhibit *M. catarrhalis* binding to Chang epithelial cells suggesting that the interactions with these cells were via cell-associated fibronectin.

The binding domains for laminin were found within the amino acid residues mentioned above. Binding assays with recombinant proteins revealed that the major binding regions were localized in the N-terminal parts, where both proteins form a globular head.

Bacterial factors mediating adherence to tissue and extracellular matrix (ECM) components are grouped together in a single family named "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMS). Since UspA1/A2both bind fibronectin and laminin, these proteins can be designated MSCRAMMS.

According to one aspect the present invention provides a peptide having sequence ID no. 1, and fragments, homologues, functional equivalents, derivatives, degenerate

or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to another aspect the present invention provides a peptide having sequence ID no. 2, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to a further aspect the present invention provides a peptide having sequence ID no. 3, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to another aspect the present invention provides a peptide having sequence ID no. 4, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to a further aspect the present invention provides a peptide having sequence ID no. 5, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to a further aspect the present invention provides a peptide having sequence ID no. 6, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to another aspect the present invention provides a peptide having sequence ID no. 7, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to another aspect the present invention provides a peptide having sequence ID no. 8, and fragments, homologues, functional equivalents, derivatives, degenerate

or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to another aspect the present invention provides a peptide having sequence ID no. 9, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to another aspect the present invention provides a peptide having sequence ID no. 10, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

According to another aspect, the present invention provides use of at least one peptide according to the invention for the production of a medicament for the treatment or prophylaxis of an infection, preferably an infection caused by *M. catarrhalis*, in particular caused by carriage of *M. catarrhalis* on mucosal surfaces.

According to another aspect, the invention further provides a ligand comprising a fibronectin binding domain, said ligand consisting of an amino acid sequence selected from the group consisting of Sequence ID No. 1, Sequence ID No. 2 and Sequence ID No. 3, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

The invention further provides a ligand comprising a laminin binding domain, said ligand consisting of an amino acid sequence selected from the group consisting of Sequence ID No. 4 to Sequence ID No. 8, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

Further, the present invention provides a ligand comprising a C3 or C3met binding domain, said ligand

consisting of an amino acid sequence selected from the group consisting of Sequence ID No. 4, Sequence ID No. 6, Sequence ID No. 9 and Sequence ID No. 10, and fragments, homologues, functional equivalents, derivatives, degenerate or hydroxylation, sulphonation or glycosylation products and other secondary processing products thereof.

Further, the present invention provides a medicament comprising one or more ligands according to the invention and one or more pharmaceutically acceptable adjuvants, vehicles, excipients, binders, carriers, or preservatives.

The present invention further provides a vaccine comprising one or more ligands according to the present invention and one or more pharmaceutically acceptable adjuvants, vehicles, excipients, binders, carriers, or preservatives.

The present invention also provides a method of treating or preventing an infection in an individual, preferably an infection caused by *M. catarrhalis*, in particular caused by carriage of *M. catarrhalis* on mucosal surfaces, comprising administering a pharmaceutically effective amount of a medicament or vaccine according to the present invention.

The present invention also provides a nucleic acid sequence encoding a ligand, protein or peptide of the present invention, as well as homologues, polymorphisms, degenerates and splice variants thereof.

The present invention further provides a peptide consisting of Sequence ID No. 2, or a fragment, homologue, functional equivalent, or hydroxylation, sulphonation or glycosylation product thereof which retain fibronectin binding properties.

The present invention also provides a peptide consisting of Sequence ID No. 3, or a fragment, homologue, functional

equivalent, or hydroxylation, sulphonation or glycosylation product thereof which retain fibronectin binding properties.

The present invention provides a ligand comprising a fibronectin binding domain, said ligand consisting of an amino acid sequence selected from the group consisting of Sequence ID No. 2 or Sequence ID No. 3, or a fragment, homologue, functional equivalent, or hydroxylation, sulphonation or glycosylation product thereof which retain fibronectin binding properties.

The present invention further provides a polypeptide or a polypeptide truncate comprising at least one of the conserved sequences of Sequence ID No. 2 or Sequence ID No. 3 or a fragment, homologue, functional equivalent, or hydroxylation, sulphonation or glycosylation product thereof with the ability of binding fibronectin.

Further disclosure of the aspects, problems, solutions and features of the present invention will be apparent from the following detailed description of the invention with reference to the drawings and the appended claims.

The expression ligand as it is used herein is intended to denote both the whole molecule which binds to the receptor and any part thereof which includes the receptor binding domain such that it retains the receptor binding

property. Ligands comprising equivalent receptor binding domains are also included in the present invention.

The expressions fragment, homologue, functional equivalent and derivative relate to variants, modifications and/or parts of the peptides and protein fragments according to the invention which retain the desired fibronectin, laminin, C3 or C3met binding properties.

A homologue of UspA1 according to the present invention is defined as a sequence having at least 72% sequence identity, as can be seen from table 1 below.

A fragment according to the present invention is defined as any of the homologue sequences which are truncated or extended by 1, 2, 5, 10, 15, 20 amino acids at the N-terminus and/or truncated or extended by 1, 2, 5, 10, 15, 20 amino acids at the C-terminus.

The expressions degenerate, hydroxylation, sulphonation and glycosylation products or other secondary processing products relate to variants and/or modifications of the peptides and protein fragments according to the invention which have been altered compared to the original peptide or protein fragment by degeneration, hydroxylation, sulphonation or glycosylation but which retain the desired fibronectin, laminin, C3 or C3met binding properties.

The present invention concerns especially infections caused by Moraxella catarrhalis. A peptide according to the present invention can be used for the treatment or prophylaxis of otitis media, sinusitis or lower respiratory tract infections.

Table 1: Multiple alignment of full length UspA1 protein sequences, associated identity percentages

	O12E	O35E	O46E	P44	TTA24	TTA37	V1171
ATCC25238	81	75	83	83	84	79	84
O12E		74	77	83	76	72	75
O35E			72	74	83	73	78
O46E				81	81	82	80

P44					81	75	77
TTA24						76	84
TTA37							78

Table 2: UspA2 Pileup Analysis - Strains and sequences used

	acc	Strain	*des*	*sl*
TREMBL:O54407_MORCA	O54407	O35E	Ubiquitous surface protein A 2.	576
TREMBL:Q58XP4_MORCA	Q58XP4	MC317	UspA2.	650
TREMBL:Q848S1_MORCA	Q848S1	E22	Ubiquitous surface protein A2H.	877
TREMBL:Q848S2_MORCA	Q848S2	V1122	Ubiquitous surface protein A2.	616
TREMBL:Q8GH86_MORCA	Q8GH86	P44	UspA2.	668
TREMBL:Q9L961_MORCA	Q9L961	TTA37	USPA2H.	889
TREMBL:Q9L962_MORCA	Q9L962	O46E	USPA2H.	894
TREMBL:Q9L963_MORCA	Q9L963	O12E	USPA2 (Ubiquitous surface protein A2).	684
TREMBL:Q9XD51_MORCA	Q9XD51	V1171	UspA2.	674
TREMBL:Q9XD53_MORCA	Q9XD53	TTA24	UspA2.	613
TREMBL:Q8RTB2_MORCA	Q8RTB2	SP12-5	UspA2	686
TREMBL:Q9XD55_MORCA	Q9XD55	ATCC25238	UspA2.	630
Forsgren_UspA2			UspA2.	630

Accordingly, the present invention provides a ligand isolated from *Moraxella catarrhalis* outer membrane protein which has laminin and/or fibronectin and/or C3-binding, wherein said ligand is a polypeptide comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1-10 which are derived from the full-length *Moraxella catarrhalis* BC5 UspA1 & UspA2 sequences shown below, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation,

sulphonation or glycosylation product or other secondary processing product thereof.

Full-length UspA1 from *Moraxella catarrhalis* strain BC5:

MNKIYKVKKN AAGHLVACSE FAKGHTKKAV LGSLLIVGIL GMATTASAQK
VGKATNKISG GDNNTANGTY LTIGGGDYNK TKGRYSTIGG GLFNEATNEY
STIGSGGYNK AKGRYSTIGG GGYNEATNQY STIGGGDNNT AKGRYSTIGG
GGYNEATIEN STVGGGGYNQ AKGRNSTVAG GYNNEATGTD STIAGGRKNQ
ATGKGSFAAG IDNKANADNA VALGNKNTIE GENSVAIGSN NTVKKGQQNV
FILGSNTDTT NAQNGSVLLG HNTAGKAATI VNSAEVGGLS LTGFAGASKT
GNGTVSVGKK GKERQIVHVG AGEISDTSTD AVNGSQLHVL ATVVAQNKAD
IKDLDDEVGL LGEEINSLEG EIFNNQDAIA KNQADIKTLE SNVEEGLLDL
SGRLLDQKAD IDNNINNIYE LAQQQDQHSS DIKTLKNNVE EGLLDLSGRL
IDQKADLTKD IKALESNVEE GLLDLSGRLI DQKADIAKNQ ADIAQNQTDI
QDLAAYNELQ DAYAKQQTEA IDALNKASSA NTDRIATAEL GIAENKKDAQ
IAKAQANENK DGIAKNQADI QLHDKKITNL GILHSMVARA VGNNTQGVAT
NKADIAKNQA DIANNIKNIY ELAQQQDQHS SDIKTLAKVS AANTDRIAKN
KAEADASFET LTKNQNTLIE QGEALVEQNK AINQELEGFA AHADVQDKQI
LQNQADITTN KTAIEQNINR TVANGFEIEK NKAGIATNKQ ELILQNDRLN
RINETNNHQD QKIDQLGYAL KEQGQHFNNR ISAVERQTAG GIANAIAIAT
LPSPSRAGEH HVLFGSGYHN GQAAVSLGAA GLSDTGKSTY KIGLSWSDAG
GLSGGVGGSY RWK

Full-length UspA2 from *Moraxella catarrhalis* strain BC5:

MKTMKLLPLK IAVTSAMIIG LGAASTANAQ AKNDITLEDL PYLIKKIDQN
ELEADIGDIT ALEKYLALSQ YGNILALEEL NKALEELDED VGWNQNDIAN
LEDDVETLTK NQNAFAEQGE AIKEDLQGLA DFVEGQEGKI LQNETSIKKN
TQRNLVNGFE IEKNKDAIAK NNESIEDLYD FGHEVAESIG EIHAHNEAQN
ETLKGLITNS IENTNNITKN KADIQALENN VVEELFNLSG RLIDQKADID
NNINNIYELA QQQDQHSSDI KTLKKNVEEG LLELSDHIID QKTDIAQNQA
NIQDLATYNE LQDQYAQKQT EAIDALNKAS SENTQNIEDL AAYNELQDAY
AKQQTEAIDA LNKASSENTQ NIEDLAAYNE LQDAYAKQQA EAIDALNKAS
SENTQNIAKN QADIANNITN IYELAQQQDK HRSDIKTLAK TSAANTDRIA
KNKADDDASF ETLTKNQNTL IEKDKEHDKL ITANKTAIDA NKASADTKFA
ATADAFTKNG NAITKNAKSI TDLGTKVDGF DSRVTALDTK VNAFDGRITA

LDSKVENGMA AQAALSGLFQ PYSVGKFNAT AALGGYGSKS AVAIGAGYRV NPNLAFKAGA AINTSGNKKG SYNIGVNYEF

In a preferred embodiment, the ligand is a polypeptide [or polypeptide truncate compared with a wild-type polypeptide] comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1-10, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

The term ligand is used herein to denote both the whole molecule which binds to laminin and/or fibronectin and/or C3 and any part thereof which includes a laminin and/or fibronectin and/or C3-binding domain such that it retains the respective binding property. Thus "ligand" encompasses molecules which consist only of the laminin and/or fibronectin and/or C3-binding domain i.e. the peptide region or regions required for binding.

For the purposes of this invention laminin, fibronectin or C3-binding properties of a polypeptide can be ascertained as follows:

For the purposes of this invention laminin, fibronectin or C3-binding properties of a polypeptide can be ascertained as follows: Polypeptides can be labelled with ^{125}Iodine or other radioactive compounds and tested for binding in radio immunoassays (RIA) as fluid or solid phase (e.g., dot blots). Moreover, polypeptides can be analysed for binding with enzyme-linked immunosorbent assays (ELISA) or flow cytometry using appropriate antibodies and detection systems. Interactions between polypeptides and laminin, fibronectin, or C3 can further be examined by surface plasmon resonance (Biacore). Examples of methods are exemplified in detail in the Material and Methods section.

In another preferred embodiment, the polypeptide [or polypeptide truncate compared with a wild-type polypeptide]

comprises or consists of at least one of the conserved sequences from within SEQ ID NO: 1-10 which are identified in the alignment shown herein. Hence, in this embodiment, the polypeptide [or polypeptide truncate compared with a wild-type polypeptide] comprises of consists of at least one of:

From UspA1 (conserved fragments from the fibronectin binding domain - '/' separating alternative choices of an amino acid at a position)

G T/V V S V G S/K Q/E/K/A G/N K/N/G/H/S E R Q I V N/H V G A G Q/N/E/K I S/R A/D T/D S T D A V N G S Q L H/Y A L A S/K/T T/A/V I/V

S T D A V N G S Q L

L L N/D L S G R L L/I D Q K A D I D N N I N N/H I Y E/D L A Q Q Q D Q H S S D I K T L K

D Q K A D I D N N I N

L A Q Q Q D Q H S S D I K T L K

From UspA2 (conserved fragments from the fibronectin binding domain - '/' separating alternative choices of an amino acid at a position)

K A D I D N N I N N/H I Y E L A Q Q Q D Q H S S D

I K/Q T/A L K/E K/N/S N V/I E/V E G/E L L/F E/N L S D/G H/R I/L I D Q K T/A D I/L A/T Q/K N/D

From UspA2 (conserved fragments from the C3-binding domain - '/' separating alternative choices of an amino acid at a position)

I E/Q D L A A Y N E L Q D A Y A K Q Q A/T E A I D A L N K A S S E N T Q N I A K N Q A D I A N N I T/N N I Y E L A Q Q Q D K/Q H R/S S D I K T L A K T/A S A A N T D/N R I

D L A A Y N E L Q D A Y A K Q Q

E A I D A L N K A S S E N T Q N I A K N Q A D I A N N I

It will be understood that the polypeptide ligands of the invention can comprise a laminin and/or fibronectin and/or C3-binding domain of sequence recited herein which is modified by the addition or deletion of amino acid residues to or from the sequences recited herein at either or both the N or C termini, which modified peptides retain the ability to bind laminin and/or fibronectin and/or C3, respectively. Accordingly, the invention further provides a ligand comprising or consisting of a polypeptide in which 50, 40, 30, 20, 10, 5, 3 or 1 amino acid residues have been added to or deleted from an amino acid sequence recited herein at either or both the N or C termini, wherein said modified polypeptide retains the ability to bind laminin and/or fibronectin and/or C3; and/or elicit an immune response against the non-modified peptide. By extension it is meant lengthening the sequence using the context of the peptide from the full-length amino acid sequence from which it is derived.

As regards fragments of the polypeptides of the invention, any size fragment may be used in the invention (based on the homologue sequences/conserved regions/functional domatins discussed herein) provided that the fragment retains the ability to bind laminin and/or fibronectin and/or C3. It may be desirable to isolate a minimal peptide which contains only those regions required for receptor binding.

Polypeptide ligands according to the invention may be derived from known *Moraxella catarrhalis* UspA1 or UspA2 proteins by truncation at either or both of the N- and C-termini. Truncates are not the full-length native UspA1 or

A2 molecules. Accordingly, the invention further provides a wild-type UspA1 sequence lacking at least (or exactly) 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160 etc to 298 amino acids from the N-terminus, and/or lacking at least (or exactly) 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, 180, 200 etc to 450 amino acids from the C-terminus. Preferably, the truncate retains fibronectin binding function (optionally also laminin and/or C3-binding).

Table 3. Possible combinations of truncations to the N- and C- termini of wild-type UspA1 protein.

No. of amino acids lacking, at least or exactly:																												
From the N-terminus	From the C-terminus																											
0	X	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	340	360	380	400	420	440	450	
20	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
30	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
40	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
50	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
60	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
70	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
80	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
100	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
120	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
140	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
160	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
180	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
200	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
220	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
240	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
260	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
280	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	
298	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	320	240	360	380	400	420	440	450	

Accordingly the invention further provides a wild-type UspA2 sequence lacking at least (or exactly) 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, 164 amino acids from the N-terminus, and/or lacking at least (or exactly) 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 180, 200 etc to 312 amino acids from the C-terminus. Preferably, the truncate retains fibronectin binding function (optionally also laminin and/or C3-binding). Possible truncates may be selected from those shown in the following table, all of which are within the scope of the invention.

Table 4. Possible combinations of truncations to the N- and C- termini of wild-type UspA2 protein

No. of amino acids lacking, at least or exactly																				
From the N-terminus	From the C-terminus																			
0	X	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
20	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
30	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
40	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
50	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
60	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
70	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
80	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
100	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
120	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
140	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
160	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312
164	0	20	30	40	50	60	70	80	100	120	140	160	180	200	220	240	260	280	300	312

Accordingly the invention further provides a wild-type UspA2 sequence lacking at least (or exactly) 5, 10, 15, 20, 25 or 29 amino acids from the N-terminus, and/or lacking at least (or exactly) 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, 180, 200 etc to 453 amino acids from the C-terminus. Preferably, the truncate retains laminin binding function (optionally also fibronectin and/or C3-binding). Possible truncates may be selected from those shown in the following table, all of which are within the scope of the invention.

Table 5. Possible combinations of truncations to the N- and C- termini of wild-type UspA2 protein

No. of amino acids lacking, at least or exactly:							
From the C-terminus	**From the N-terminus**						
0	X	5	10	15	20	25	29
20	0	5	10	15	20	25	29
30	0	5	10	15	20	25	29
40	0	5	10	15	20	25	29
50	0	5	10	15	20	25	29
60	0	5	10	15	20	25	29
70	0	5	10	15	20	25	29
80	0	5	10	15	20	25	29
100	0	5	10	15	20	25	29
120	0	5	10	15	20	25	29
140	0	5	10	15	20	25	29
160	0	5	10	15	20	25	29
180	0	5	10	15	20	25	29
200	0	5	10	15	20	25	29
220	0	5	10	15	20	25	29
240	0	5	10	15	20	25	29
260	0	5	10	15	20	25	29
280	0	5	10	15	20	25	29
300	0	5	10	15	20	25	29
320	0	5	10	15	20	25	29
340	0	5	10	15	20	25	29
360	0	5	10	15	20	25	29
380	0	5	10	15	20	25	29

400	0	5	10	15	20	25	29
420	0	5	10	15	20	25	29
440	0	5	10	15	20	25	29
453	0	5	10	15	20	25	29

Accordingly the invention further provides a wild-type UspA2 sequence lacking (or exactly) 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160 etc. to 301 amino acids from the N-terminus, and/or lacking at least (or exactly) 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160 or 172 amino acids from the C-terminus. Preferably, the truncate retains C3 binding function (optionally also fibronectin and/or laminin binding). Possible truncates may be selected from those shown in the following table, all of which are within the scope of the invention.

Table 6. Possible combinations of truncations to the N- and C- termini of wild-type UspA2 protein

No. of amino acids lacking, at least or exactly:													
From the N-terminus	**From the C-terminus**												
0	X	20	30	40	50	60	70	80	100	120	140	160	172
20	0	20	30	40	50	60	70	80	100	120	140	160	172
30	0	20	30	40	50	60	70	80	100	120	140	160	172
40	0	20	30	40	50	60	70	80	100	120	140	160	172
50	0	20	30	40	50	60	70	80	100	120	140	160	172
60	0	20	30	40	50	60	70	80	100	120	140	160	172
70	0	20	30	40	50	60	70	80	100	120	140	160	172
80	0	20	30	40	50	60	70	80	100	120	140	160	172
100	0	20	30	40	50	60	70	80	100	120	140	160	172
120	0	20	30	40	50	60	70	80	100	120	140	160	172
140	0	20	30	40	50	60	70	80	100	120	140	160	172
160	0	20	30	40	50	60	70	80	100	120	140	160	172
180	0	20	30	40	50	60	70	80	100	120	140	160	172

200	0	20	30	40	50	60	70	80	100	120	140	160	172
220	0	20	30	40	50	60	70	80	100	120	140	160	172
240	0	20	30	40	50	60	70	80	100	120	140	160	172
260	0	20	30	40	50	60	70	80	100	120	140	160	172
280	0	20	30	40	50	60	70	80	100	120	140	160	172
290	0	20	30	40	50	60	70	80	100	120	140	160	172
301	0	20	30	40	50	60	70	80	100	120	140	160	172

Known wild-type UspA1 sequences that may be truncated in this way are those of strains ATCC25238 (MX2; GenBank accession no. AAD43465), P44 (AAN84895), O35E (AAB96359), TTA37 (AAF40122), O12E (AAF40118), O46E (AAF36416), V1171 (AAD43469), TTA24 (AAD43467) (see Table 1/Figure 19); or BC5 (see above). Known wild-type UspA2 sequences that may be truncated in this way are those of strains O35E (GenBank accession no. O4407), MC317 (GenBank accession no. Q58XP4), E22 (GenBank accession no. Q848S1), V1122 (GenBank accession no. Q848S2), P44 (GenBank accession no. Q8GH86), TTA37 (GenBank accession no. Q9L961), O46E (GenBank accession no. Q9L962), O12E (GenBank accession no. Q9L963), V1171 (GenBank accession no. Q9XD51), TTA24 (GenBank accession no. Q9XD53), SP12-5 (GenBank accession no. Q8RTB2), ATCC25238 (GenBank accession no. Q9XD55) (see Table 2/Figure 20); or BC5 [Forsgren_UspA2] (see above).

Ideally the UspA1 or UspA2 truncate of this embodiment comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 1-10 or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof; or comprises or consists of at least one of the conserved sequences from within these regions which are identified in the alignment shown in herein, for example:

From UspA1 (conserved fragments from the fibronectin binding domain - '/' separating alternative choices of an amino acid at a position)

G T/V V S V G S/K Q/E/K/A G/N K/N/G/H/S E R Q I V N/H V G A G Q/N/E/K I S/R A/D T/D S T D A V N G S Q L H/Y A L A S/K/T T/A/V I/V

S T D A V N G S Q L

L L N/D L S G R L L/I D Q K A D I D N N I N N/H I Y E/D L A Q Q Q D Q H S S D I K T L K

D Q K A D I D N N I N

L A Q Q Q D Q H S S D I K T L K

From UspA2 (conserved fragments from the fibronectin binding domain - '/' separating alternative choices of an amino acid at a position)

K A D I D N N I N N/H I Y E L A Q Q Q D Q H S S D

I K/Q T/A L K/E K/N/S N V/I E/V E G/E L L/F E/N L S D/G H/R I/L I D Q K T/A D I/L A/T Q/K N/D

From UspA2 (conserved fragments from the C3-binding domain - '/' separating alternative choices of an amino acid at a position)

I E/Q D L A A Y N E L Q D A Y A K Q Q A/T E A I D A L N K A S S E N T Q N I A K N Q A D I A N N I T/N N I Y E L A Q Q Q D K/Q H R/S S D I K T L A K T/A S A A N T D/N R I

D L A A Y N E L Q D A Y A K Q Q

E A I D A L N K A S S E N T Q N I A K N Q A D I A N N I

It may be convenient to produce fusion proteins containing polypeptide ligands as described herein. Accordingly, in a further embodiment, the invention provides fusion proteins comprising polypeptide ligands according to the invention. Preferably a fusion protein according to this embodiment is less than 50% identical to any known fully length sequence over its entire length. Such fusions can constitute a derivative of the polypeptides of the invention. Further derivatives can be the use of the polypeptides of the invention to as a carrier to covalently couple peptide or saccharide moieties. They may be coupled for instance to pneumococcal capsular oligosaccharides or polysaccharides, or *Moraxella catarrhalis* lipooligo-saccaharides, or non-typeable *Haemophilus influenzae* lipooligosaccaharides.

Homologous peptides of the invention may be identified by sequence comparison. Homologous peptides are preferably at least 60% identical, more preferably at least 70%, 80%, 90%, 95% or 99% identical in ascending order of preference to the peptide sequence disclosed herein or fragments thereof or truncates of the invention over their entire length. Preferably the homologous peptide retains the ability to bind fibronectin and/or laminin and/or C3; and/or elicit an immune response against the peptide sequences disclosed herein or fragment thereof.

Figures 19 and 20 show an alignment of peptide sequences of UspA1 and UspA2 of different origin which indicates regions of sequence that are capable of being modified to form homologous sequences whilst retained function (i.e. fibronectin and/or laminin and/or C3 binding ability). Homologous peptides to the BC5 SEQ ID NO: 1-10 peptides are for instance those sequences corresponding to the BC5 sequence from other strains in Figures 19 and 20.

Vaccines of the Invention

The polypeptides /peptides /functional domains /homologues /fragments /truncates /derivatives of the invention should ideally be formulated as a vaccine comprising an effective amount of said component(s) and a pharmaceutically acceptable excipient.

The vaccines of the invention can be used for administration to a patient for the prevention or treatment of *Moraxella catarrhalis* infection or otitis media or sinusitis or lower respiratory tract infections. They may be administered in any known way, including intramuscularly, parenternally, mucosally and intranasally.

Combination Vaccines of the Invention

The vaccines of the present invention may be combined with other *Moraxella catarrhalis* antigens for prevention or treatment of the aforementioned diseases.

The present inventors have found in particular that *Moraxella catarrhalis* has at least 2 means of hampering the host immune system from attacking the organism. In addition to the interaction with C3 (and C4BP) mentioned in the Examples below, *M. catarrhalis* has a strong affinity for soluble and membrane bound human IgD through protein MID (also known as OMP106). Moraxella-dependent IgD-binding to B lymphocytes results in a polyclonal immunoglobulin synthesis which may prohibit production of specific monoclonal anti-moraxella antibodies. The fact that M. catarrhalis hampers the human immune system in several ways might explain why M. catarrhalis is such a common inhabitant of the respiratory tract.

The inventors believe that the combination of antigens involved in the IgD-binding function (MID) and C3-binding function (UspA1 and/or UspA2) can provide an immunogenic composition giving the host enhanced defensive capabilities against Moraxella's hampering of the human immune system thus providing an enhanced decrease in M. catarrhalis carriage on mucosal surfaces.

A further aspect of the invention is therefore a vaccine composition comprising an effective amount of UspA1 and/or UspA2 (particularly the latter) (for instance full-length polypeptides or polypeptides /peptides /functional domains /homologues /fragments /truncates /derivatives of the invention as described herein, preferably which retains a C3-binding function) in combination with an effective amount of protein MID (for instance full-length polypeptides or polypeptides /peptides /functional domains /homologues /fragments /truncates /derivatives thereof, preferably which retain a human IgD-binding function), and a pharmaceutically acceptable excipient.

Protein MID, and IgD-binding homologous/fragments/truncates thereof is described in WO 03/004651 (incorporated by reference herein). Particularly suitable fragments for this purpose is a polypeptide comprising (or consisting of) the F2 fragment described in WO 03/004651, or sequences with at least 60, 70, 80, 90, 95, 99% identity thereto which preferably retain human IgD-binding activity.

The MID and UspA components of this combination vaccine may be separate from each other, or may be conveniently fused together by known molecular biology techniques.

Brief description of the drawings

Figure 1 shows thirteen M. catarrhalis strains tested for fibronectin binding (A). Strong fibronectin binding correlated to UspA1/A2 expression as detected by anti-UspA1/A2 pAb (B-I). Flow cytometry profiles of M. catarrhalis BBH18 wild type and UspA1/A2 deficient mutants show an UspA1/A2-dependent binding to soluble fibronectin. The profiles of wild type clinical isolate (B and F) and corresponding mutants devoid of UspA1 (C and G), or UspA2 (D and H), and double mutants (E and I) lacking both UspA1 and UspA2 are shown. Bacteria were incubated with rabbit anti-UspA1/A2 or fibronectin followed by an anti-fibronectin pAb. FITC-conjugated rabbit pAb was subsequently added followed

by flow cytometry analysis. A typical experiment out of three with the mean fluorescence intensity (MFI) for each profile is shown.

Figure 2 shows that *M. catarrhalis* RH4 UspA2 deficient mutants do not bind ^{125}I-labeled fibronectin. *E. coli* BL21 was included as a negative control not binding fibronectin. Bacteria were incubated with ^{125}I-labeled fibronectin followed by several washes and analyzed in a gamma counter. Fibronectin binding to the RH4 wild type expressing both UspA1 and A2 was set as 100 %. The mean values of three independent experiments are shown. Error bars represent standard deviations (SD). Similar results were obtained with *M. catarrhalis* BBH18.

Figure 3 shows pictures that verify that *M. catarrhalis* mutants devoid of UspA1 and UspA2 do not bind to immobilized fibronectin. *M. catarrhalis* wild type was able to adhere at a high density on fibronectin coated glass slides (A). *M. catarrhalis* Δ*uspA1* mutant was also retained at a high density (B), whereas *M. catarrhalis* Δ*uspA2* and Δ*uspA1/A2* double mutants adhered poorly (*C* and *D*). Glass slides were coated with fibronectin and incubated with *M. catarrhalis* RH4 and its corresponding UspA1/A2 mutants. After several washes, bacteria were Gram stained.

Figure 4 is a graph showing that recombinant UspA1 and A2 bind to fibronectin in a dose-dependent manner. Specific fibronectin binding is shown for UspA1$^{50-770}$ and UspA2$^{30-539}$. Both UspA proteins (40 nM) were coated on microtiter plates and incubated with increasing concentrations of fibronectin followed by detection with rabbit anti-human fibronectin pAb and HRP-conjugated anti-rabbit pAb. Mean values of three separate experiments are shown and error bars indicate SD.

Figure 5. The active fibronectin binding domains for UspA1 and UspA2 are located between amino acids 299 to 452 and 165 to 318, respectively. Truncated proteins derived from UspA1 (*A*) and UspA2 (*B*) are shown. All fragments were

tested for fibronectin binding in ELISA. Forty nM of each truncated fragment was coated on microtiter plates and incubated with 80 µg/ml and 120 µg/ml fibronectin for UspA1 and UspA2, respectively. Bound fibronectin was detected with rabbit anti-fibronectin pAb followed by HRP-conjugated anti-rabbit pAb. Results are representative for three sets of experiments. Error bars represent SD.

Figure 6 shows the sequence according to sequence ID No. 1, and the sequence homology between $UspA1^{299-452}$ and $UspA2^{165-318}$. The 31 identical amino acid residues are within brackets.

Figure 7 shows that truncated $UspA1^{50-491}$ and $UspA1^{299-452}$ fragments competitively inhibit *M. catarrhalis* UspA-dependent fibronectin binding. *M. catarrhalis ΔuspA1/A2* double mutants, which do not bind fibronectin, were included as negative controls. UspA1 recombinant proteins were pre-incubated with 2 mg/100 ml fibronectin before incubation with *M. catarrhalis*. The mean fluorescence values (MFI) of *M. catarrhalis* with bound fibronectin detected by FITC conjugated anti-fibronectin pAb in flow cytometry are shown. $UspA1^{50-491}$ and $UspA1^{299-452}$ resulted in 95 % and 63 % inhibition respectively. Error bars represent mean ± SD of three independent experiments.

Figure 8 shows that $UspA1^{299-452}$ and $UspA2^{165-318}$ inhibit *M. catarrhalis* adherence to Chang conjunctival cells via cell-associated fibronectin. Chang epithelial cells expressed fibronectin on the surface as revealed by an anti-fibronectin pAb and flow cytometry (A). Pre-incubation with the fibronectin binding proteins $UspA1^{299-452}$, $UspA2^{165-318}$, or anti-fibronectin pAb resulted in significantly reduced binding by *M. catarrhalis* RH4 as compared to control recombinant proteins ($UspA1^{433-580}$ and $UspA2^{30-177}$) and a control antibody (anti-ICAM1 mAb) (B). $P<0.05$ by two-tailed

paired Student's *t* test. Mean values of three separate experiments are shown and error bars indicate SD.

Fig. 9A shows binding of *M. catarrhalis* RH4 to laminin via UspA1 and A2. *M. catarrhalis* RH4 wild type (*wt*) strongly bound to immobilized laminin with a mean OD of 1.27. RH4*ΔuspA1* showed mean OD of 1.14 (89.8 % of the wild type). RH4*ΔuspA2* and the double mutant RH4*ΔuspA1/A2* had a mean OD of 0.19 and 0.23 respectively (15.0% and 18.1% of the wild type). This was not significantly different from the residual adhesion to bovine serum albumin coated plates. Thirty µg/ml of laminin or bovine serum albumin were coated on microtiter plates. They were blocked followed by incubation with bacteria suspension and finally washed. Bound bacteria was detected with anti-MID pAb and HRP-conjugated anti-rabbit pAb. The mean results of 3 representative experiments are shown. Error bars represent standard deviations (SD).

Fig. 9B shows the binding of recombinant UspA1 and A2 laminin in a dose-dependent manner. Specific laminin binding is shown for UspA1$^{50-770}$ and UspA2$^{30-539}$.Both UspA proteins (40 nM) were coated on microtiter plates and incubated with increasing concentrations of laminin followed by detection with rabbit anti-laminin pAb and HRP-conjugated anti-rabbit pAb. Mean values of three separate experiments are shown and error bars indicate SD.

Fig. 10 A and B show that the active laminin binding domains for UspA1$^{50-770}$ (*A*) and UspA2$^{30-539}$ (*B*) are located in the N-terminal halves. Forty nM of recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ together with the truncated proteins were coated on microtiter plates and incubated with 20 µg/ml of laminin followed by detection with rabbit anti-laminin pAb and HRP-conjugated anti-rabbit pAb. Mean values of three separate experiments are shown and error bars indicate SD.

Fig. 11 is a schematic illustration of C3, covalent bound C3b and C3met. (A) The C3-molecule in serum consists

of one α-chain and one β-chain. (B) The α-chain contains an internal thioester site that after activation can attach covalently to a microbial surface. (C) The C3 has been treated with methylamine, which becomes covalently attached to the thioester.

Fig. 12 illustrates that *M. catarrhalis* counteracts the classical and alternative pathways of the complement system by the outer membrane proteins UspA1 and A2. (A) *M. catarrhalis* RH4 wild-type (wt), the Δ*uspA1*, the Δ*uspA2* or the Δ*uspA1/A2* mutants were incubated in the presence of 10 % NHS. (B) The Δ*uspA1/A2* mutant was incubated with 10 % NHS supplemented with either EDTA or Mg-EGTA. Bacteria were collected at the indicated time points. After overnight incubation, colony forming units (cfu) were counted. The number of bacteria at the initiation of the experiments was defined as 100 %. Mean values of three separate experiments are shown and error bars indicate S.D. (A) The mean values after 5 min for the Δ*uspA1*, the Δ*uspA2* or the Δ*uspA1/A2* mutants were significantly different from the wild-type ($P < 0.05$). (B) The mean values after 5 min for the Δ*uspA1/A2* mutant and after 10 min for the Δ*uspA1/A2* mutant incubated Mg-EGTA were significantly different from the wild-type ($P < 0.05$).

Fig. 13 illustrates that *Moraxella catarrhalis* binds C3 in serum independently of complement activation. Flow cytometry profiles showing C3 binding to (A) *M. catarrhalis* RH4 or (B) *Streptococcus pneumoniae*. Bacteria were incubated with NHS or NHS pretreated with EDTA. Thereafter, a rabbit anti-human C3d pAb and as a seconddary layer a FITC-conjugated goat anti-rabbit pAb were added followed by flow cytometry analysis. Bacteria in the absence of NHS, but in the presence of both pAb, were defined as background fluorescence. One representative experiment out of three is shown.

Fig. 14 illustrates that *M. catarrhalis* non-covalently binds purified methylamine-treated C3 in a dose-dependent manner, and that the binding is based on ionic interactions. Flow cytometry profiles showing (A) binding with increasing concentrations of C3met. (B) The mean fluorescence intensity (mfi) of each profile in panel (A) is shown. (C) C3met binding of RH4 decreases with increasing concentrations of NaCl. Bacteria were incubated with C3met with or without NaCl as indicated. C3met binding was measured by flow cytometry as described in Figure 3. Error bars indicate SD. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Fig. 15 illustrates that flow cytometry profiles of *M. catarrhalis* RH4 wild type and UspA1/A2 deficient mutants show a UspA1/UspA2-dependent C3met/ C3 binding. The profiles of a wild type clinical isolate (A, F, K) and corresponding mutants devoid of protein MID (B, G, L), UspA1 (C, H, M), UspA2 (D, I, N), or both UspA1 and UspA2 (E, J, O) are shown. Bacteria were incubated with C3met (A-E), NHS-EDTA (F-J) or NHS (K-O) and detected as outlined in Figure 3. One typical experiment out of three with the mean fluorescence intensity (mfi) for each profile is shown.

Fig. 16 illustrates that C3met binds to purified recombinant UspA2$^{30-539}$, whereas only a weak C3met binding to UspA1$^{50-770}$ is observed. Furthermore, the C3met binding region of UspA2 was determined to be located between the amino acid residues 200 to 458. (A) The recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ were immobilized on a nitrocellulose membrane. The membrane was incubated with [^{125}I]-labelled C3met overnight and bound protein was visualized with a Personal FX (Bio-Rad) using intensifying screens. The recombinant protein MID$^{962-1200}$ was included as a negative control. (B) UspA1$^{50-770}$, UspA2$^{30-539}$ and a series of truncated UspA2 proteins were coated on microtiter plates and incubated with C3met, followed by incubation with goat anti-human C3 pAb and HRP-conjugated anti-goat pAb. The mean values out of three

experiments are shown. The background binding was subtracted from all samples. Error bars correspond to S.D. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Fig. 17 illustrates that addition of recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ to serum inhibit C3b deposition and killing of *M. catarrhalis* via the alternative pathway. Flow cytometry profiles show C3b-deposition on RH4Δ*uspA1/A2* after incubation with (A) NHS or NHS preincubated with recombinant (rec.) UspA1$^{50-770}$ and UspA2$^{30-539}$, or (B) NHS-Mg-EGTA or NHS-Mg-EGTA preincubated with UspA1$^{50-770}$ and UspA2$^{30-539}$. After addition of the various NHS combinations, bacteria were analyzed as described in Figure 13. (C) RH4Δ*uspA1/A2* was incubated with 10 % NHS or NHS-Mg-EGTA. For inhibition, the NHS-Mg-EGTA was incubated with 100 nM UspA1$^{50-770}$ and/ or UspA2$^{30-539}$ before addition of bacteria. Bacteria were collected at the indicated time points. The number of bacteria at the initiation of the experiments was defined as 100 %. Mean values of three separate experiments are shown and error bars indicate S.D. The time points 10, 20 and 30 min for the Δ*uspA1/A2* mutant preincubated with recombinant proteins were significantly different from the Δ*uspA1/A2* mutant incubated with Mg-EGTA alone ($P < 0.05$).

Fig. 18 illustrates that recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ decrease haemolysis of rabbit erythrocytes by inhibition of the alternative pathway. NHS was incubated with or without 100 nM UspA1$^{50-770}$ and/ or UspA2$^{30-539}$ at 37 °C for 30 min. NHS at the indicated concentrations was thereafter added to rabbit erythrocytes. After incubation for 30 min, the suspensions were centrifuged and the supernatants were measured by spectrophotometry. Maximum haemolysis in each experiment was defined as 100 %. Mean values of three separate experiments are shown and error bars correspond to S.D. The results obtained with NHS + UspA2$^{30-539}$ and NHS + UspA1$^{50-770}$/ UspA2$^{30-539}$ at NHS

concentrations of 2, 3 and 4% were significantly different from the NHS control ($P < 0.05$).

Fig. 19 illustrates a pileup-analysis of UspA1 for eight different strains, to show the homology of different parts of UspA1.

Fig. 20 illustrates a pileup-analysis of UspA2 for thirteen different strains to show the homology of different parts of UspA2.

Fig. 21 illustrates %identity in regions identified on Forsgren sequence computed as the ratio between the number of exact matches and the length of the region alignment, where the region alignment is that part of the above total alignment containing the Forsgren region.

Materials and methods

Interaction between *M. catarrhalis* and fibronectin

Bacterial strains and culture conditions

The sources of the clinical *M. catarrhalis* strains are listed in table 7. *M. catarrhalis* BBH18 and RH4 mutants were constructed as previously described.[23, 58] The *M. catarrhalis* strains were routinely cultured in brain heart infusion (BHI) liquid broth or on BHI agar plates at 37 °C. The UspA1-deficient mutants were cultured in BHI supplemented with 1.5 µg/ml chloramphenicol (Sigma, St. Louis, MO), and UspA2-deficient mutants were incubated with 7 µg/ml zeocin (Invitrogen, Carlsbad, CA). Both chloramphenicol and zeocin were used for growth of the double mutants.

Table 7. Clinical strains of *M. catarrhalis* used in the present study

Strain	Clinical Source	Reference
BBH18	Sputum	[53]
D1	Sputum	[53]
Ri49	Sputum	[53]
C10	Sputum	[10]
F16	Sputum	[10]

Bro2	Respiratory tract	[53]
Z14	Pharynx	[10]
S6-688	Nasopharynx	[23]
Bc5	Nasopharynx	[20]
RH4	Blood	[53]
RH6	Blood	[53]
R14	Unknown	[10]
R4	Unknown	[10]
SÖ-1914	Tympanic cavity aspirate	[23]

Note: The strains C10, R4 did not have the *uspA1* gene, whereas F16, R14, Z14 lacked the *uspA2* gene.[10] The remaining strains contained both *uspA1* and *A2* genes (data not shown).

DNA method

To detect the presence *uspA1, A2, and A2H* genes in those strains which this was unknown, primers and PCR conditions as described by Meier et al. was used.[50] Partial sequencing was also carried out with the $UspA1^{299-452}$ and $UspA2^{165-318}$ 5′ and 3′ primers of the respective *uspA1* and *uspA2* gene of RH4 and BBH18. Confirmation of the presence of the amino acid residues "DQKADIDNNINNIYELAQQQDQHSSDIKTLK" was also performed by PCR with a primer (5′-CAAAGCTGACATCCAAGCACTTG-3′) designed from the 5′ end of this sequence and 3′ primers for *uspA1* and *A2* as described by Meier at al.[50]

Recombinant proteins construction and expression

Recombinant $UspA1^{50-770}$ and $UspA^{230-539}$, which are devoid of their hydrophobic C-termini, has recently been described.[58] The genomic DNA was extracted from *M. catarrhalis* Bc5 using a DNeasy tissue kit (Qiagen, Hilden, Germany). In addition, recombinant proteins corresponding to multiple regions spanning $UspA1^{50-770}$ *and* $UspA2^{30-539}$ were also constructed by the same method. The primers used are listed in table 8. All constructs were sequenced according to standard methods. Expression and purification of the

recombinant proteins were done as described previously.[59] Proteins were purified using columns containing a nickel resin (Novagen) according to the manufacturer's instructions for native conditions. The recombinant proteins were analyzed on SDS-PAGE as described.[21]

Table 8. Primers used in this present study

Protein	5′ primer	3′ primer
UspA1$^{50-770}$	gcgtctgcggatccagtaggcaaggcaacc	ccctgaagctttagtgcataacctaattg
UspA1$^{50-491}$	gcgtctgcggatccagtaggcaaggcaacc	ttgagcaagcttagcttggtttttagcg
UspA1$^{50-197}$	gcgtctgcggatccagtaggcaaggcaacc	acctgtggcaagcttcttcctgcc
UspA1$^{50-321}$	gcgtctgcggatccagtaggcaaggcaacc ggtgtcactaagcttacctgcaccaacatgaac	
UspA1$^{299-452}$	ggatttgcaggtgcatcggatcctggtaatggtact	gtcttttgtaagatcaagcttttgatcaat
UspA1$^{433-580}$	catagctctgatatggatccacttaaaaac	catgctgagaagcttacctagattgg
UspA1$^{557-704}$	gccaaagcacaagcggatccaaataaagac	ggtcttattggtagtaagcttagcttggttttg
UspA1$^{680-770}$	gttgagcaaaaggatcccatcaatcaagag	ccctgaagctttagtgcataacctaattg
UspA2$^{30-539}$	cgaatgcggatcctaaaaatgatataactttagagg	cattaagcttggtgtctaatgcagttac
UspA2$^{30-177}$	cgaatgcggatcctaaaaatgatataactttagagg	ctcatgaccaaaatcaagcttatcttcgatagactc
UspA2$^{101-240}$	gatattgcggatccggaagatgatgttgaaac	gatcaataagcttaccgcttagattgaatagttcttc
UspA2$^{101-318}$	gatattgcggatccggaagatgatgttgaaac	gtcaatcgcttcaagcttcttttgagcatactg
UspA2$^{165-318}$	gagattgagaaggatccagatgctattgct	gtcaatcgcttcaagcttcttttgagcatactg
UspA2$^{302-458}$	gctcaaaaccaagcggatccccaagatctg	ggtgagcgtttcaagctttgcatcagcatcggc
UspA2$^{446-539}$	gcaagtgctgcggatcctgatcgtattgct	cattaagcttggtgtctaatgcagttac

Antibodies

Rabbit anti-UspA1/A2 polyclonal antibodies (pAb) were recently described in detail.[58] The other antibodies used were rabbit anti-human fibronectin pAb, swine FITC-conjugated anti-rabbit pAb, swine horseradish peroxidase (HRP) conjugated anti-rabbit pAb and finally a mouse anti-human CD54 (ICAM1) monoclonal antibody (mAb). Antibodies were from Dakopatts (Glostrup, Denmark).

Flow cytometry analysis

The UspA1/A2-protein expression and the capacity of *M. catarrhalis* to bind fibronectin were analyzed by flow cytometry. *M. catarrhalis* wild type strains and UspA1/A2-deficient mutants were grown overnight and washed twice in phosphate buffered saline containing 3 % fish gelatin (PBS-gelatin). The bacteria (10^8) were then incubated with the anti-UspA1/A2 antiserum or 5 µg fibronectin (Sigma, St Louis, MO). They were then washed and incubated for 30 min at room temperature (RT) with FITC-conjugated anti-rabbit pAb (diluted according to the manufacturer's instructions) or with 1/100 dilution of rabbit anti-human fibronectin pAb (if fibronectin was first added) for 30 min at RT before incubation with the FITC-conjugated anti-rabbit pAb. After three additional washes, the bacteria were analyzed by flow cytometry (EPICS, XL-MCL, Coulter, Hialeah, FL). All incubations were kept in a final volume of 100 µl PBS-gelatin and the washings were done with the same buffer. Anti-fibronectin pAb and FITC-conjugated anti-rabbit pAb were added separately as a negative control for each strain analyzed. Fibronectin inhibition studies were carried out by pre-incubating 0.25 µmoles of UspA fragments for 1 h with 2 µg of fibronectin before incubation with *M. catarrhalis* bacteria (10^8). The residual free amount of fibronectin that bound to *M. catarrhalis* was determined by flow cytometry as outlined above.

Binding of *M. catarrhalis* to immobilized fibronectin

Glass slides were coated with 30 µl aliquots of fibronectin (1 mg/ml) and air dried at RT. After washing once with PBS, the slides were incubated in Petri dishes with pre-chilled bacteria at late exponential phase (optical density (OD) at 600 nm = 0.9). After 2 h at RT, glass slides were washed once with PBS followed by Gram staining.

Protein labeling and radio immunoassay (RIA)

Fibronectin was ^{125}Iodine labeled (Amersham, Buckinghamshire, England) to a high specific activity (0.05 mol iodine per mol protein) with the Chloramine T method.[21] *M. catarrhalis* strains BBH18 and RH4 together with their corresponding mutants were grown overnight on solid medium and were washed in PBS with 2 % bovine serum albumin (BSA). Bacteria (10^8) were incubated for 1 h at 37°C with ^{125}I-labeled fibronectin (1600 kcpm/sample) in PBS containing 2 % BSA. After three washings with PBS 2 % BSA, ^{125}I-labeled fibronectin bound to bacteria was measured in a gamma counter (Wallac, Espoo, Finland).

Enzyme-linked immunosorbent assay (ELISA)

Microtiter plates (Nunc-Immuno Module; Roskilde, Denmark) were coated with 40 nM of purified recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ proteins in 75 mM sodium carbonate, pH 9.6 at 4 °C overnight. Plates were washed four times with washing buffer (50 mM Tris-HCl, 0.15 M NaCl, and 0.1 % Tween 20, pH 7.5) and blocked for 2 h at RT with washing buffer containing 3 % fish gelatin. After four additional washings, the wells were incubated for 1 h at RT with fibronectin (120 µg/ml) diluted in three-fold step in 1.5 % fish gelatin (in wash buffer). Thereafter, the plates were washed and incubated with rabbit anti-human fibronectin pAb for 1 h. After additional washings, HRP-conjugated anti-rabbit pAb was added and incubated for 1 h at RT. Both the antihuman fibronectin and HRP-conjugated anti-rabbit pAb were diluted 1:1,000 in washing buffer containing 1.5 % fish gelatin. The wells were washed four times and the plates were developed and measured at OD_{450}. ELISAs with truncated proteins spanning UspA1$^{50-770}$ and UspA2$^{30-539}$ were performed with fixed doses of fibronectin at 80 µg/ml and 120 µg/ml, respectively.

Cell line adherence inhibition assay

Chang conjunctival cells (ATCC CCL 20.2) were cultured in RPMI 1640 medium (Gibco BRL, Life Technologies, Paisley,

Scotland) supplemented with 10 % fetal calf serum, 2 mM L-glutamine, and 12 µg of gentamicin/ ml. On the day before adherence inhibition experiments, cells were harvested, washed twice in gentamicin-free RPMI 1640, and added to 96 well tissue culture plates (Nunc) at a final concentration of 10^4 cells/ well in 200 µl of gentamicin-free culture medium. Thereafter, cells were incubated overnight at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air. On the day of experiments, inhibition of *M. catarrhalis* adhesion was carried out by pre-incubating increasing concentration of recombinant UspA1/A2 truncated proteins containing the fibronectin binding domains (UspA1$^{299-452}$ and UspA2$^{165-318}$) or rabbit anti-human fibronectin pAb (diluted 1:50) for 1 h. Nonfibronectin binding recombinant proteins (UspA1$^{433-580}$ and UspA2$^{30-177}$) were used as controls. Chang epithelial cells are known to express ICAM1.[18] Hence an anti-ICAM1 antibody was used to differentiate if the inhibitory effect of the anti-fibronectin antibody was secondary to steric hindrance. Subsequently, *M. catarrhalis* RH4 (10^6) in PBS-gelatin was inoculated onto the confluent monolayers. In all experiments, tissue culture plates were centrifuged at 3,000 x g for 5 min and incubated at 37 °C in 5 % CO_2. After 30 min, infected monolayers were rinsed several times with PBS-gelatin to remove non-adherent bacteria and were then treated with trypsin-EDTA (0.05 % trypsin and 0.5 mM EDTA) to release the Chang cells from the plastic support. Thereafter, the resulting cell/ bacterium suspension was seeded in dilution onto agar plates containing BHI and incubated overnight at 37 °C in 5 % CO_2.

Determination of fibronectin expression in Chang conjunctival epithelial cells

Chang conjunctival epithelial cells were harvested by scraping followed by re-suspension in PBS-gelatin. Cells (1 x 10^6 /ml) were labeled with rabbit anti-human fibronectin pAb followed by washing and incubation with a FITC-

conjugated anti-rabbit pAb. After three additional washes, the cells were analyzed by flow cytometry as outlined above.

Interaction between *M. catarrhalis* and laminin

Bacterial strains and culture conditions

The clinical *M. catarrhalis* strains BBH18 and RH4 and their corresponding mutants were previously described.[58] Both strains have a relatively higher expression of UspA2 compared to UspA1.[58] The mutants expressed equal amount of *M. catarrhalis* immunoglobulin D-binding protein (MID) when compared to wild type strains. Bacteria were routinely cultured in brain heart infusion (BHI) broth or on BHI agar plates at 37 °C. The UspA1-deficient, UspA2-deficient and double mutants were cultured in BHI supplemented with antibiotics as described.[58]

Recombinant protein construction and expression Recombinant $UspA1^{50-770}$ and $UspA2^{30-539}$, which are devoid of their hydrophobic C-termini, were manufactured.[58] In addition, recombinant proteins corresponding to multiple regions spanning $UspA1^{50-770}$ and $UspA2^{30-539}$ were used.[78]

Antibodies

Rabbit anti-UspA1/A2 and anti-MID polyclonal antibodies (pAb) were used.[22, 58] Rabbit anti-laminin pAb was from Sigma (St Louis, MO, USA). Swine horseradish peroxidase (HRP)-conjugated anti-rabbit pAb was from Dakopatts (Glostrup, Denmark).

Binding of *M. catarrhalis* to immobilized laminin

Microtiter plates (Nunc-Immuno Module; Roskilde, Denmark) were coated with Engelbreth-Holm-Swarm mouse sarcoma laminin (Sigma, Saint Louis, USA) or bovine serum albumin (BSA) (30 µg/ml) in Tris-HCL, pH 9.0 at 4°C overnight. The plates were washed with phosphate buffered saline and 0.05% Tween 20, pH 7.2 (PBS-Tween) and subsequently blocked with 2 % BSA in PBS + 0.1 % Tween 20, pH 7.2. *M. catarrhalis* RH4 and BBH18 (10^8) in 100 µl were then added followed by incubation for 1 h. Unbound bacteria

were removed by washing 3 times with PBS-Tween. Residual bound bacteria were detected by means of an anti-MID pAb, followed by detection with HRP-conjugated anti-rabbit pAb. The plates were developed and measured at OD_{450} according to a standard protoccol.

Enzyme-linked immunosorbent assay (ELISA)

Microtiter plates (Nunc-Immuno Module) were coated with 40 nM of purified recombinant $UspA1^{50-770}$ and $UspA2^{30-539}$ proteins in 75 mM sodium carbonate, pH 9.6 at 4°C. Plates were washed four times with washing buffer (50 mM Tris-HCl, 0.15 M NaCl, and 0.1 % Tween 20, pH 7.5) and blocked at RT with washing buffer containing 3 % fish gelatin. After additional washings, the wells were incubated for 1 h at RT with laminin at different dilutions as indicated in 1.5 % fish gelatin (in wash buffer). Thereafter, the plates were washed and incubated with rabbit anti-laminin pAb. After additional washings, HRP-conjugated anti-rabbit pAb was added and incubated at RT. Both the anti-laminin and HRP-conjugated anti-rabbit pAb were diluted 1:1,000 in washing buffer containing 1.5 % fish gelatin. The wells were washed and the plates were developed and measured at OD_{450}. Uncoated wells incubated with identical dilutions of laminin were used as background controls. ELISAs with truncated proteins spanning $UspA1^{50-770}$ and $UspA2^{30-539}$ were performed with fixed doses of laminin (20 µg/ml).

Interaction between *M. catarrhalis* and C3 and C3met

Bacterial strains and culture conditions

The clinical *M. catarrhalis* isolates and related subspecies have recently been described in detail.[21, 53] Type strains were from the Culture Collection, University of Gothenburg (CCUG; Department of Clinical Bacteriology, Sahlgrenska Hospital, Gothenburg, Sweden), or the American Type Culture Collection (ATCC; Manassas, Va); *Neisseria gonorrheae* CCUG 15821, *Streptococcus pyogenes* CCUG 25570 and 25571, *Streptococcus agalactiae* CCUG 4208, *Streptococcus pneumoniae*

ATCC 49619, *Legionella pneumophila* ATCC 33152, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 29213, and finally *Staphylococcus aureus* ATCC 25923. The remaining strains in Table 9 were clinical isolates from Medical Microbiology, Department of Laboratory Medicine, Malmö University Hospital, Lund University, Sweden.

Table 9. *M. catarrhalis* is a unique C3/ C3met binding bacterium. Related moraxella subspecies and other common human pathogens do not bind C3/ C3met (mfi < 2.0). After incubation with EDTA-treated NHS or C3met, bacteria were analysed by flow cytometry using a rabbit anti-C3d pAb and a FITC-conjugated goat anti-rabbit pAb.

Species	NHS-EDTA (mfi)	C3met (mfi)
Moraxella catarrhalis RH4	8.7	22.1
M. osloensis	< 2.0	< 2.0
M. bovis	< 2.0	< 2.0
M. caniculi	< 2.0	< 2.0
M. nonliquefacie	< 2.0	< 2.0
N. pharyngis	< 2.0	< 2.0
N. sicca	< 2.0	< 2.0
N. flava	< 2.0	< 2.0
N. subflava	< 2.0	< 2.0
Oligella ureolytica (n=2)	< 2.0	< 2.0
Haemophilus influenzae (n=7)	< 2.0	< 2.0
Streptococcus pneumoniae (n=11)	< 2.0	< 2.0
Legionella pneumophila (n=2)	< 2.0	< 2.0
Pseudomonas aeruginosa (n=2)	< 2.0	< 2.0
Listeria monocytogenes	< 2.0	< 2.0
Yersinia entercolitica	< 2.0	< 2.0
Staphylococcus aureus (n=3)	< 2.0	< 2.0
Streptococcus pyogenes (n=2)	< 2.0	< 2.0
Streptococcus agalactia	< 2.0	< 2.0
Enterococcus faecalis	< 2.0	< 2.0
Helicobacter pylori	< 2.0	< 2.0
Escherichia coli (n=2)	< 2.0	< 2.0
M. ovis	< 2.0	< 2.0
M. caviae	< 2.0	< 2.0
Neisseria gonorrheae	< 2.0	< 2.0
N. meningtidis	< 2.0	< 2.0
N. mucosa	< 2.0	< 2.0

The different non-moraxella species were grown on appropriate standard culture media. *M. catarrhalis* strains were routinely cultured in brain heart infusion (BHI) liquid broth or on BHI agar plates at 37°C. *M. catarrhalis* BBH18 and RH4 mutants were manufactured as previously described.[22, 23, 58] The MID-deficient mutants were grown in BHI containing 50 µg/ml kanamycin. The UspA1-deficient mutants were cultured in BHI supplemented with 1.5 µg/ml chloramphenicol (Sigma, St. Louis, MO), and UspA2-deficient mutants were incubated with 7 µg/ml zeocin (Invitrogen, Carlsbad, CA). Both chloramphenicol and zeocin were used for growth of the UspA1/ A2 double mutants.

Antibodies

Rabbits were immunized intramuscularly with 200 µg recombinant full-length UspA1 emulsified in complete Freunds adjuvant (Difco, Becton Dickinson, Heidelberg, Germany), and boosted on days 18 and 36 with the same dose of protein in incomplete Freunds adjuvant.[22] Blood was drawn 3 weeks later. To increase the specificity, the anti-UspA1 antiserum was affinity-purified with Sepharose-conjugated recombinant UspA1$^{50-770}$.[58] The antiserum bound equally to UspA1 and UspA2 and was thus designated anti-UspA1/ A2 pAb. The rabbit anti-human C3d pAb and the FITC-conjugated swine anti-rabbit pAb were purchased from Dakopatts (Glostrup, Denmark), and the goat anti-human C3 were from Advanced Research Technologies (San Diego, CA). The horseradish peroxidase (HRP)-conjugated donkey anti-goat pAb was obtained from Serotec (Oxford, UK).

Proteins and iodine labelling

The manufacture of recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$, which are devoid of their hydrophobic C-termini, has recently been described.[23] The truncated UspA1 and UspA2 proteins were manufactured as described in detail by Tan *et al.*[78] C3b was purchased from Advanced Research Technologies. C3(H_2O) was obtained by freezing and thawing

of purified C3. The C3b-like molecule (C3met) was made by incubation of purified C3 with 100 mM methylamine (pH 8.0) for 2 h at 37°C, and subsequent dialysis against 100 mM Tris-HCl (pH 7.5), 150 mM NaCl. For binding studies, C3met was labelled with 0.05 mol ^{125}I (Amersham, Buckinghamshire, England) per mol protein, using the Chloramine T method.[25]

Flow cytometry analysis

Binding of C3 to *M. catarrhalis* and other species was analyzed by flow cytometry. Bacteria were grown on solid medium overnight and washed twice in PBS containing 2 % BSA (Sigma) (PBS-BSA). Thereafter, bacteria (10^8 colony forming units; cfu) were incubated with C3met, C3b, C3(H_2O), or 10 % NHS with or without 10 mM EDTA or 4 mM $MgCl_2$ and 10 mM EGTA (Mg-EGTA) in PBS-BSA for 30 min at 37°C. After washings, the bacteria were incubated with anti-human C3d pAb for 30 min on ice, followed by washings and incubation for another 30 min on ice with FITC-conjugated goat anti-rabbit pAb. After three additional washes, bacteria were analyzed by flow cytometry (EPICS, XL-MCL, Coulter, Hialeah, FL). All incubations were kept in a final volume of 100 µl PBS-BSA and the washings were done with the same buffer. The anti-human C3d pAb and FITC-conjugated anti-rabbit pAb were added separately as a negative control for each strain analyzed. In the inhibition studies, serum was preincubated with 100 nM of the recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ proteins for 30 min at 37°C. To analyze the characteristics of the *M. catarrhalis* and C3 interaction, increasing concentrations of NaCl (0 - 1.0 M) was added to bacteria and C3met. To analyze UspA1/ A2 expression, bacteria (10^8 cfu) were incubated with the anti-UspA1/ A2 pAb and washed as described above. A FITC-conjugated goat anti-rabbit pAb diluted according to the manufacturers instructions was used for detection. To assure that EDTA did not disrupt the outer membrane proteins UspA1 and UspA2, *M. catarrhalis* was incubated with or without EDTA followed by detection of UspA1/ A2 expression.

EDTA, at the concentrations used in the NHS-EDTA experiments, did not change the density of UspA1/A2.

Serum and serum bactericidal assay

Normal human serum (NHS) was obtained from five healthy volunteers. The blood was allowed to clot for 30 min at room temperature and thereafter incubated on ice for 60 min. After centrifugation, sera were pooled, aliquoted and stored at -70°C. To inactivate both the classical and alternative pathways, 10 mM EDTA was added. In contrast, Mg-EGTA was included to inactivate the classical pathway. Human serum deficient in the C4BP was prepared by passing fresh serum through a HiTrap column (Amersham Biosciences) coupled with mAb 104, a mouse mAb directed against CCP1 of the α-chain of C4BP.[41] The flow through was collected and the depleted serum was stored in aliquots at -70°C. Serum depleted of C1q was obtained via the first step of C1q purification [79] using Biorex 70 ion exchange chromatography (Bio-Rad, Hercules, CA). The resulting sera displayed normal haemolytic activity. The factor D and properdin deficient serum was kindly provided by Dr. Anders Sjöholm (Department of Medical Microbiology, Lund University, Lund, Sweden). *M. catarrhalis* strains were diluted in 2.5 mM Veronal buffer, pH 7.3 containing 0.1 % (wt/vol) gelatin, 1 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 2.5 % dextrose ($DGVB^{++}$). Bacteria (10^3 cfu) were incubated together with 10 % NHS and EDTA or Mg-EGTA in a final volume of 100 µl. The bacteria/ NHS was incubated at 37°C and at various time points, 10 µl aliquots were removed and spread onto BHI agar plates. In inhibition studies, 10 % serum was incubated with 100 nM of the recombinant $UspA1^{50-770}$ and $UspA2^{30-539}$ proteins for 30 min at 37°C before bacteria were added.

Dot blot assays

Purified recombinant $UspA1^{50-770}$ and $UspA2^{30-539}$ diluted in three-fold steps (1.9 - 150 nM) in 100 µl of 0.1 M Tris-HCl, pH 9.0 were applied to nitrocellulose membranes (Schleicher

& Schüll, Dassel, Germany) using a dot blot device. After saturation, the membranes were incubated for 2 h with PBS-Tween containing 5 % milk powder at room temperature and washed four times with PBS-Tween. Thereafter, 5 kcpm [^{125}I]-labelled C3met in PBS-Tween with 2 % milk powder was added overnight at 4°C. The bound protein was visualized with a Personal FX (Bio-Rad) using intensifying screens.

Surface plasmon resonance (Biacore)

The interaction between UspA1$^{50-770}$ or UspA2$^{30-539}$ and C3 was further analysed using surface plasmon resonance (Biacore 2000; Biacore, Uppsala, Sweden) as recently described for the UspA1/2-C4BP interaction.[58] The K_D (the equilibrium dissociation constant) was calculated from a binding curve showing response at equilibrium plotted against the concentration using steady state affinity model supplied by Biaevaluation software (Biacore).

Enzyme-linked immunosorbent assay (ELISA)

Microtiter plates (Nunc-Immuno Module; Roskilde, Denmark) were coated with triplets of purified recombinant UspA1$^{50-770}$, UspA2$^{30-539}$, or the truncated UspA1 and UspA2 fragments (40 nM in 75 mM sodium carbonate, pH 9.6) at 4°C overnight. Plates were washed four times with washing buffer (PBS with 0.1 % Tween 20, pH 7.2) and blocked for 2 hrs at room temperature with washing buffer supplied with 1.5 % ovalbumin (blocking buffer). After washings, the wells were incubated overnight at 4°C with 0.25 µg C3met in blocking buffer. Thereafter, the plates were washed and incubated with goat anti-human C3 in blocking buffer for 1 h at RT. After additional washings, HRP-conjugated donkey anti-goat pAbs was added for another 1 h at RT. The wells were washed four times and the plates were developed and measured at OD_{450}.

Haemolytic assay

Rabbit erythrocytes were washed three times with ice-cold 2.5 mM Veronal buffer, pH 7.3 containing 0.1 % (wt/vol)

gelatin, 7 mM $MgCl_2$, 10 mM EGTA, and 2.5 % dextrose (Mg^{++}EGTA), and resuspended at a concentration of 0.5 x 10^9 cells/ml. Erythrocytes were incubated with various concentrations (0 to 4 %) of serum diluted in Mg^{++}EGTA. After 1 h at 37°C, erythrocytes were centrifuged and the amount of lysed erythrocytes was determined by spectrophotometric measurement of released hemoglobin at 405 nm. For inhibition with UspA1 and UspA2, 10 % serum was preincubated with 100 nM of recombinant UspA1$^{50-770}$ and/ or UspA2$^{30-539}$ proteins for 30 min at 37°C, and thereafter added to the erythrocytes at 0 to 4 %.

Isolation of polymorphonuclear leukocytes and phagocytosis

Human polymorphonuclear leukocytes (PMN) were isolated from fresh blood of healthy volunteers using macrodex (Pharmalink AB, Upplands Väsby, Sweden). The PMN were centrifuged for 10 min at 300g, washed in PBS and resuspended in RPMI 1640 medium (Life Technologies, Paisley, Scotland). The bacterial suspension (0.5 x 10^8) was opsonized with 3 % of either NHS or NHS-EDTA, or 20 µg of purified C3met for 15 min at 37°C. After washes, bacteria were mixed with PMN (1 x 10^7 cells/ml) at a bacteria/PMN ratio of 10:1 followed by incubation at 37°C with end-over-end rotation. Surviving bacteria after 0, 30, 60, and 120 min of incubation was determined by viable counts. The number of engulfed NHS-treated bacteria was compared with bacteria phagocytosed in the absence of NHS. *S. aureus* opsonized with NHS was used as positive control.

Examples and results

Interaction between *M. catarrhalis* and fibronectin

M. catarrhalis devoid of UspA1 and A2 does not bind soluble or immobilized fibronectin.

We selected a random series of *M. catarrhalis* clinical strains (n=13) (table 7) and tested them for fibronectin binding in relation to their UspA1/A2 expression by flow cytometry analysis. High UspA1/A2 expression as determined

by high mean fluorescence intensity (MFI) was correlated to UspA1/A2 expression (Pearson correlation coefficient 0.77, $P<0.05$) (figure 1*A*). However, to discriminate between UspA1 and A2 expression was not possible with our anti-UspA1/A2 pAb. Moreover, the presence of UspA2H protein contributing to the binding was unlikely as the *uspA2H* gene was not found in the strains used in this study (data not shown).

Two *M. catarrhalis* isolates (BBH18 and RH4) and their specific mutants lacking UspA1, UspA2 or both proteins were also analyzed by flow cytometry. *M. catarrhalis* BBH18 strongly bound fibronectin with a mean fluorescence intensity (MFI) of 96.1 (figure 1*F*). In contrast, BBH18*ΔuspA1* showed a decreased fibronectin binding with an MFI of 68.6 (figure 1*G*). Fibronectin binding to BBH18Δ*uspA2* and the double mutant BBH18*ΔuspA1/A2* revealed an MFI of only 10.7 and 11.5, respectively (figure 1*H*, 1*I*). Similar results were obtained with UspA1/A2 mutants of the clinical strain *M. catarrhalis* RH4. Taken together, these results suggest that UspA1 and A2 bound fibronectin and that the ability of the bacteria to bind fibronectin strongly depended on UspA1/A2 expression.

To further analyze the interaction between fibronectin and *M. catarrhalis*, ^{125}I-labeled fibronectin was incubated with two clinical *M. catarrhalis* isolates (BBH18 and RH4) and their respective mutants. The wild type *M. catarrhalis* RH4 strongly bound ^{125}I-fibronectin while the corresponding *ΔuspA1* mutant showed 80 % binding of the wild type. In contrast, the *ΔuspA2* and double mutant bound ^{125}I-fibronectin at 14 % and 12 %, respectively, which was just above the background levels (5.0 to 10 %) (figure 2). Similar results were obtained with *M. catarrhalis* BBH18 and the corresponding UspA1/A2 mutants. Thus, our results suggest that both UspA1 and A2 are required for the maximal binding of soluble fibronectin by *M. catarrhalis*.

To investigate the bacterial attachment to immobilized fibronectin, *M. catarrhalis* RH4 and its corresponding *ΔuspA1/A2* mutants were applied onto fibronectin coated glass slides. After 2 h of incubation, slides were washed, and subsequently Gram stained. *M. catarrhalis* wild type and the *ΔuspA1* mutant were found to strongly adhere to the fibronectin coated glass slides (figure 3*A* and 3*B*). In contrast, *M. catarrhalis ΔuspA2* and *ΔuspA1/A2* double mutants weakly adhered to the fibronectin coated glass slide with only a few bacteria left after washing (figure 3*C* and 3*D*, respectively). Experiments with another *M. catarrhalis* clinical isolate (BBH18) and its derived mutants showed a similar pattern indicating that UspA2 was of major importance for *M. catarrhalis* binding to immobilized fibronectin.

The fibronectin binding domains include amino acid residues located between 299 and 452 of UspA1 and between 165 and 318 of UspA2

To further analyze the interactions of UspA1 and A2 with fibronectin, truncated $UspA1^{50-770}$ and $UspA2^{30-539}$ were recombinantly produced in *E. coli*, coated on microtiter plates and incubated with increasing concentrations of fibronectin. Bound fibronectin was detected with an anti-human fibronectin pAb followed by incubation with a horseradish peroxidase conjugated anti-rabbit pAb. Both recombinant $UspA1^{50-770}$ and $UspA2^{30-539}$ bound soluble fibronectin and the interactions were dose-dependent (figure 4).

To define the fibronectin-binding domain of UspA1, recombinant proteins spanning the entire molecule of $UspA1^{50-770}$ were manufactured. Fibronectin was incubated with the immobilized UspA1 proteins fragments and the interactions were quantified by ELISA. $UspA1^{50-491}$ bound fibronectin almost as efficiently as $UspA1^{50-770}$ suggesting that the binding domain was within this part of the protein. Among the other

truncated fragments, UspA1$^{299-452}$ efficiently bound fibronectin (figure 5*A*). In parallel, the interactions between fibronectin and several recombinant UspA2 fragments including amino acids UspA2$^{30-539}$ were analyzed. The two fragments UspA2$^{101-318}$ and UspA2$^{165-318}$ strongly bound fibronectin (figure 5*B*). Our findings provide significant evidence that the binding domains include residues found within UspA1$^{299-452}$ and UspA2$^{165-318}$. A sequence comparison between these two binding fragments revealed that the 31 amino acid residues "DQKADIDNNINNIYELAQQQDQHSSDIKTLK" were identical for UspA1 and A2 (figure 6). Moreover, this repeat sequence was also found in the *uspA1* and *A2* gene of *M. catarrhalis* BBH18 and RH4 (data not shown).

UspA1$^{50-491}$ and UspA1$^{299-452}$ fragments competitively inhibit *M. catarrhalis* fibronectin binding

To further validate our findings on the UspA1/A2 fibronectin binding domains, recombinant truncated UspA1 proteins were tested for their capacity to block fibronectin binding to *M. catarrhalis*. Fibronectin (2 µg) was pre-incubated with 0.25 µmoles of recombinant UspA1 fragments and subsequently incubated with *M. catarrhalis*. Finally, *M. catarrhalis* UspA-dependent fibronectin binding was measured by flow cytometry. Pre-incubation with UspA1$^{50-491}$ and UspA1$^{299-452}$ resulted in decreased fibronectin binding with a 95 % reduction for UspA1$^{50-491}$ and a 63 % reduction for UspA1$^{299-452}$ (figure 7). When fibronectin was pre-incubated with the truncated UspA2$^{101-318}$, an inhibition of 50% was obtained.

Thus, the fibronectin binding domains of UspA1 and A2 block the interactions between fibronectin and *M. catarrhalis*.

UspA1$^{299-452}$ and UspA2$^{165-318}$ inhibit *M. catarrhalis* adherence to Chang epithelial cells

Epithelial cells are known to express fibronectin and many bacteria attach to epithelial cells via cell-associated

fibronectin.[46, 54, 69, 77] Previous studies have shown that *M. catarrhalis* adhere to epithelial cells.[43, 49] We analyzed Chan conjunctival cells, which have frequently been used in adhesion experiments with respiratory pathogens. Chang cells strongly expressed fibronectin as revealed by flow cytometry analysis (figure 8*A*).

To analyze whether the UspA-dependent fibronectin binding was important for bacterial adhesion, Chang epithelial cells were pre-incubated with anti-human fibronectin pAb, or the recombinant proteins $UspA1^{299-452}$ and $UspA2^{165-318}$. Thereafter, *M. catarrhalis* RH4 was added and bacterial adhesion analyzed. The relative adherence (measured by the number of colony forming units) after pre-incubation with 0.4 µmoles per 200 µl of $UspA1^{299-452}$, $UspA2^{165-318}$, or an anti-human fibronectin pAb were 36 %, 35 % and 32%, respectively. Higher concentrations of recombinant peptides did not result in further inhibition. In contrast, the non-fibronectin binding fragments $UspA1^{433-580}$ and $UspA2^{30-177}$ did not inhibit the interactions between *M. catarrhalis* and the Chang epithelial cells (figure 8*B*). Thus, fibronectin on Chang epithelial cells may function as a receptor for *M. catarrhalis* and the amino acid residues 299-452 of UspA1 and 165-318 of UspA2 contain the ligand responsible for the interactions.

Interaction between *M. catarrhalis* and laminin

M. catarrhalis binds laminin through UspA1 and A2

Two clinical *M. catarrhalis* isolates (BBH18 and RH4) and their specific mutants lacking UspA1, UspA2 or both proteins were analyzed by a whole-cell ELISA. *M. catarrhalis* RH4 strongly bound to immobilized laminin.(figure 9A). In contrast, *M. catarrhalis* RH4 *uspA1* mutant (RH4*ΔuspA1*) showed a laminin binding of 89.9% of the wild type. *M. catarrhalis* RH4 *uspA2* mutant (RH4*ΔuspA2*) and the double mutant RH4*ΔuspA1/A2* 15.2% and 18.1% binding capacity of the wild type, respectively. This was not significantly different

from the residual adhesion to BSA coated plates. Similar results were obtained with UspA1/A2 mutants originating from the clinical strain *M. catarrhalis* BBH18. In these two strains (BBH18 and RH4), UspA2 is the predominant protein expressed as compared to UspA1, explaining the minimal difference in binding between the wild type and RH4Δ*uspA1*. Taken together, these results show that UspA1 and A2 bound laminin.

To further analyze the binding between UspA1/A2 and laminin, truncated UspA1$^{50-770}$ and UspA2$^{30-539}$ were produced in *E. coli*. Recombinant proteins were coated on microtiter plates and incubated with increasing concentrations of laminin. Bound laminin was detected with a rabbit anti-laminin pAb followed by incubation with an HRP-conjugated anti-rabbit pAb. Both recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ strongly bound soluble laminin and the binding was dose-dependent and saturable (figure 9B).

To define the laminin binding domains, recombinant UspA1 and A2 spanning the entire molecules were manufactured. Laminin was incubated with immobilized truncated UspA1 and A2 fragments and followed by quantification by ELISA. UspA1$^{50-491}$ bound to laminin almost as efficiently as UspA1$^{50-770}$ suggesting that the binding domain was within this part of the protein. However, among the other truncated fragments spanning this region, no other fragment appeared to bind laminin. The N-terminal part, UspA2$^{30-351}$, was able to retain 44.7 % binding capacity as compared to the full length protein. The shorter protein UspA2$^{30-177}$ showed a 43.7 % binding capacity. (figure 10B). These results show that the binding domains include residues found within the N-terminals of both UspA1 and UspA2.

Interaction between *M. catarrhalis* and C3 and C3met

M. catarrhalis outer membrane proteins UspA1 and UspA2 inhibit both the classical and the alternative pathway of the complement cascade

UspA2 surface expression is crucial for *M. catarrhalis* survival in normal human serum (NHS) [1, 58], i.e., moraxella UspA2 deficient mutants are rapidly killed when exposed to NHS. We have recently shown that both UspA1 and A2 bind C4BP and thus might inhibit the classical pathway of complement activation [58]. To further shed light on *M. catarrhalis* interactions with the complement system, survival of UspA1/A2 double mutants was studied in serum treated with either EGTA with addition of $MgCl_2$ (Mg-EGTA) or EDTA. Mg-EGTA inhibits the classical and lectin pathways and thus allows separate analysis of the alternative pathway. In contrast, EDTA inhibits all complement pathways by absorbing divalent cations (Mg^{2+} and Ca^{2+}). The *M. catarrhalis* RH4 wild type survived after 30 min of incubation, whereas RH4Δ*uspA1/A2* double mutant was killed by intact NHS after 10 min (Fig. 12). When the classical pathway was inhibited (NHS + Mg-EGTA), the RH4Δ*uspA1/A2* mutant survived for a significantly longer period of time as compared to NHS without any chelators, but not as long as the wild type bacterium. Furthermore, when both the classical and alternative pathways were blocked with EDTA, *M. catarrhalis* RH4Δ*uspA1/A2* survived. A similar pattern was obtained with the *M. catarrhalis* BBH18 isolate and the corresponding BBH18 Δ*uspA1/A2* mutants (not shown). In parallel, experiments with C1q and factor D/ properdin deficient sera demonstrated that both the classical and the alternative pathways were inhibited by *M. catarrhalis* (not shown). Thus, *M. catarrhalis*, a pathogen that frequently colonizes the human respiratory tract, does not only counteract the classical pathway but also the alternative pathway of the complement system by the outer membrane proteins UspA1 and A2.

M. catarrhalis absorbs C3 from EDTA-inactivated serum

C3b covalently binds to the surface of a microbe and hence induces the alternative pathway (Fig. 11*B*). To analyze whether *M. catarrhalis* can interact with C3, our RH4 wild

type strain was incubated with NHS or NHS treated with EDTA. Binding or deposition (via covalent link) of C3/ C3b at the bacterial surface of *M. catarrhalis* RH4 was detected by flow cytometry analysis with a polyclonal antibody (pAb) directed against C3d recognizing both C3 and C3b. Incubation of bacteria with NHS containing intact complement led to deposition of C3 (Fig. 13). Interestingly, when the complement cascade was inactivated in the presence of EDTA, the *M. catarrhalis* RH4 still bound C3 (Fig. 13*A*). *Streptococcus pneumoniae* that was included for comparison did not absorb C3 from the EDTA-treated serum (Fig. 13*B*). In contrast to pneumococci, *M. catarrhalis* thus bound C3 irrespectively of complement activation. The internal thioester of C3 is spontaneously hydrolysed in fluid phase to C3(H_2O). Thus, intact C3 or C3(H_2O) was the most likely forms of C3 interacting with *M. catarrhalis*. Since *M. catarrhalis* also binds C4BP [58], we wanted to exclude that C4BP was involved in the C3 binding and for that purpose we used C4BP depleted serum. *M. catarrhalis* absorbed C3 from the C4BP depleted serum to the same extent as to NHS (not shown).

Binding of C3met to *M. catarrhalis* is dose-dependent and non-covalent

Our experiments implied that C3 bound to the surface of *M. catarrhalis* irrespectively of complement activation. Therefore, we analyzed whether converted C3, which is non-functional, could bind to the bacteria. Native C3 was purified from human serum and treated with methylamine, which converts C3 to a C3met molecule equivalent to C3b without the capacity to covalently bind to microbes (Fig. 11*C*). Flow cytometry analysis revealed that the *M. catarrhalis* RH4 wild type strain efficiently bound C3met in a dose-dependent and saturable manner (Fig. 14*A* and *B*). This interaction was not mediated by the C3a part of the C3 molecule since C3b and C3(H_2O) also bound *M. catarrhalis*

(not shown). The binding between *M. catarrhalis* RH4 and C3met was based to a large extent on ionic interactions as increasing concentrations of NaCl inhibited the interaction (Fig. 14*C*). Similar results were obtained with the *M. catarrhalis* BBH18 wild type strain (not shown).

To determine whether the binding of C3 is a general feature of all *M. catarrhalis* strains, we selected a random series of clinical isolates (n=13) and analyzed their capacity to bind C3met. All *M. catarrhalis* strains bound C3met as revealed by a flow cytometry analysis with an anti-C3d pAb. The mfi values varied from 4 to 39. However, *S. pneumoniae* and *E. coli* that were included for comparison did not bind C3met.

M. catarrhalis is a unique C3 and C3met binding bacterium

To extend our analysis of bacterial C3 absorption from NHS, related moraxella subspecies (n=13) as well as common human pathogens (n=13) were incubated in the presence of NHS-EDTA. Interestingly, among all the bacterial species tested, *M. catarrhalis* was the only bacterium binding C3 in complement-inactivated serum (Table 9). All related moraxella strains as well as the other human pathogens were also analyzed for binding of C3met. In parallel with the C3 binding, *M. catarrhalis* was the only species that bound C3met. Taken together, *M. catarrhalis* has a unique feature to strongly bind C3 and C3met in a non-covalent manner.

M. catarrhalis binds C3met via the outer membrane proteins UspA1 and UspA2

To determine the *M. catarrhalis* protein responsible for the C3 binding, we tested a series of bacterial mutants devoid of the outer membrane proteins MID, UspA1 and/ or UspA2 [22, 58]. Interestingly, the binding of C3met was significantly correlated with Usp expression (Fig. 15). *M. catarrhalis* RH4Δ*mid* bound C3met to the same degree as the wild type counterpart (Fig. 15*A-B*). The RH4Δ*uspA1* mutant showed only a slightly decreased binding, whereas the

RH4Δ*uspA2* was a weaker binder as compared to the wild type counterpart (Fig. 15*C-D*). In parallel, C3met binding to the double RH4Δ*uspA1/A2* mutant was completely abolished (Fig. 15*E*). Furthermore, when the same experiments were performed using NHS-EDTA, the same pattern was seen (Fig. 15*F-J*). When normal human serum was used, all mutants showed similar amount of C3 on their surface since it was a mixture of covalent deposition and binding of C3 (Fig. 15*K-O*). Similar results were obtained with the *M. catarrhalis* BBH18 isolate and the corresponding BBH18 mutants.

To further analyze the interaction between C3 and UspA1/A2, UspA1$^{50-770}$ and UspA2$^{30-539}$ were produced in *E. coli* and purified. The recombinant proteins were dot blotted onto a nitrocellulose membrane followed by incubation with iodine-labelled C3met. Recombinant MID$^{962-1200}$, which is derived from the *M. catarrhalis* outer membrane protein MID [59], was included as a negative control. A weak binding to UspA1$^{50-770}$ was detected, whereas [^{125}I]-C3met strongly bound to UspA2$^{30-539}$ (Fig. 16*A*). These findings were further strengthened using surface plasmon resonance (i.e., Biacore). UspA1$^{50-770}$ and UspA2$^{30-539}$ were immobilized on the surface of a CM5 chip using amino coupling and C3met was injected until saturation was reached. The K_D for the interaction between C3met and UspA2$^{30-539}$ or UspA1$^{50-770}$ was 3 and 14 μM, respectively. In conclusion, we found that UspA2 was the major C3met-binding protein of *M. catarrhalis*, whereas UspA1 contributed to the binding to a lower degree. A C3 binding domain is located between amino acid residues 200 and 458 of UspA2.

To define the C3 binding domain of UspA2, recombinant proteins spanning the entire UspA2$^{30-539}$ molecule were manufactured. C3met was incubated with the immobilized full length UspA1$^{50-770}$, UspA2$^{30-539}$ and a series of truncated UspA2 proteins. Thereafter, the interactions were quantified by ELISA. In agreement with the dot blot experiments (Fig.

16*A*), UspA1$^{50-770}$ bound C3met to a much lower extent compared to UspA2$^{30-539}$ in the ELISA (Fig. 16*B*). Among the truncated protein fragments, UspA2$^{165-318}$, UspA2$^{200-539}$ and UspA2$^{302-458}$ efficiently bound C3met, suggesting that a binding domain was within the amino acid residues 200 and 458.

Recombinant UspA1/ A2 neutralizes C3 activity

In order to in detail examine the role of UspA1/A2-dependent inhibition of the alternative pathway, a series of flow cytometry experiments was performed with bacteria incubated with 10 % NHS or serum that had been preincubated with 100 nM recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$. Interestingly, a significantly decreased C3 deposition/ binding at the surface of *M. catarrhalis* RH4ΔuspA1/A2 was observed when NHS was pretreated with UspA1$^{50-770}$ and UspA2$^{30-539}$ (Fig. 17A). When the classical pathway was shut down with Mg-EGTA, similar results were obtained (Fig. 17B). Thus, the recombinant proteins UspA1$^{50-770}$ and UspA2$^{30-539}$ absorbed C3 from NHS and inhibited deposition/ binding of C3.

To determine whether absorption of C3 by recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ increased bacterial survival, the double mutant *M. catarrhalis* RH4Δ*uspA1/A2* was incubated with serum supplemented with UspA1$^{50-770}$ and UspA2$^{30-539}$ followed by determination of the number of surviving bacteria. Mg-EGTA was included in the reactions in order to inhibit the classical pathway. Interestingly, addition of recombinant UspA1$^{50-770}$ and UspA2$^{30-539}$ to NHS prevented killing of the UspA1/A2 deficient *M. catarrhalis* (Fig. 17*C*). UspA2$^{30-539}$ was most efficient in inhibiting bacterial killing as compared to UspA1$^{50-770}$. When both recombinant proteins were supplemented together, no additional inhibition of the alternative pathway was detected. Ten % NHS correspond to approximately 600 nM C3. To investigate whether more UspA1 molecules could neutralize the C3 activity, UspA1$^{50-770}$ and/ or UspA2$^{30-539}$ up to 600 nM was added. However, higher

concentrations of the recombinant proteins did not further increase the inhibition (not shown).

We also included an alternative pathway haemolytic assay consisting of rabbit erythrocytes and NHS in order to establish the role of UspA1 and A2 as inhibitors of the alternative pathway. NHS was preincubated with recombinant $UspA1^{50-770}$, $UspA2^{30-539}$, or both proteins together followed by addition to the erythrocytes. After 1 h incubation, the amount of erythrocyte lysis was determined. Interestingly, a significantly decreased haemolysis was observed when NHS was preincubated with $UspA1^{50-770}$ or $UspA2^{30-539}$ as compared to untreated NHS (Fig. 18). In parallel with the increased survival of bacteria in the presence of $UspA2^{30-539}$ or $UspA1^{50-770}$ (Fig. 17C), preincubation with $UspA2^{30-539}$ alone resulted in a more efficient inhibition of the alternative pathway as compared to when NHS was preincubated with $UspA1^{50-770}$. In conclusion, recombinant $UspA1^{50-770}$ or $UspA2^{30-539}$ interfered with the activity of the alternative pathway due to their ability to capture C3.

In addition of being a key molecule in the complement cascade, deposited C3b and iC3b (inactivated C3b) target microbes for removal in the process of opsonophagocytosis. To investigate whether C3 or C3met that was non-covalently bound at the surface of *M. catarrhalis* could still function as an opsonin, a series of phagocytosis experiments was performed. *M. catarrhalis* was preincubated with C3met, NHS or NHS treated with EDTA followed by addition of polymorphonuclear leukocytes. Interestingly, *M. catarrhalis* was not engulfed in the presence of C3met, whereas NHS strongly promoted phagocytosis (data not shown). However, when NHS was pretreated with EDTA, *M. catarrhalis* was not phagocytosed by polymorphonuclear leukocytes. Thus, C3/C3met was inactive at the *M. catarrhalis* cell surface and did not function as an opsonin.

Discussion

Interaction between *M. catarrhalis* and fibronectin

UspA1$^{299-452}$ and UspA2$^{165-318}$ from the clinical *M. catarrhalis* strain Bc5 were the shortest fragments that still bound fibronectin. Interestingly, longer fragments encompassing the amino acid sequence found within UspA1$^{299-452}$ and UspA2$^{165-318}$ displayed a more efficient binding to fibronectin (figure 5*A* and *B*). This may mean that these two regions represent partial binding domains or that the binding site is highly dependent on a specific molecular structure. UspA1$^{299-452}$ and UspA2$^{165-318}$ share a sequence of 31 identical amino acid residues including the 23 residues "NNINNIYELAQQQDQHSSDIKTL" (NNINNIY sequence). This sequence contains the epitope for the protective monoclonal antibody (mAb) 17C7 for which there is universal reactivity.[2, 50, 30] In a mouse model, passive immunization with mAb 17C7 provided protection and improved pulmonary clearance of *M. catarrhalis*.[30] It is hence most interesting that UspA1/A2 fibronectin binding domains contain these residues and argues for the importance of this region in the pathogenesis of *M. catarrhalis* respiratory tract infection.

The fibronectin binding *M. catarrhalis* BBH18 and RH4 used in our experiments also carry the 31 amino acid residues in their UspA1/A2 protein. Most *M. catarrhalis* have a part of this sequence (i.e., the NNINNIY sequence). However, strains like the O35E which has the NNINNIY sequence in their *UspA2* gene do not express a fibronectin binding UspA2 protein.[49] A likely explanation would be that the variations in the flanking regions might affect the interaction with fibronectin. Also, the conserved NNINNIY sequence itself can have minor single amino acid base changes.[28] It is thus likely that fibronectin binding would depend not just on UspA1/A2 expression, but also on the individual makeup of each UspA protein. Interestingly, an almost identical amino acid sequence can be found in the hybrid UspA2H protein with adhesive properties (*M.*

catarrhalis TTA37 and O46E).[43] This give support to our findings that the 31 amino acid sequence is important in adhesion.

In our last set of experiments, we tested whether the adherence of *M. catarrhalis* to Chang conjunctival cells could be inhibited by the fibronectin binding fragments ($UspA1^{299-452}$ and $UspA2^{165-318}$) (figure 8*B*). Preincubation with $UspA1^{299-452}$, $UspA2^{165-318}$ or an anti-fibronectin pAb resulted in decreased binding to Chang epithelial cells. These results confirm the importance of these binding domains in the interactions of UspA1/A2 with Chang epithelial cells and further suggest that fibronectin is an important receptor for UspA. In addition, it is known that FnBP facilitate the adherence of bacteria to undifferentiated and injured airways.[54, 69] Fibronectin expression by lung fibroblasts is also increased by cigarette smoke extract.[87] The role of *M. catarrhalis* UspA1/A2 binding to ECM fibronectin or epithelial cell-associated fibronectin is thus of great importance in patients with COPD and may explain the common occurrence of *M. catarrhalis* infection in this group of patients.[40]

In conclusion, we have shown that UspA1/A2 of *M. catarrhalis* BBH18, RH4 and Bc5 are crucial FnBP. Both recombinant UspA1 and A2 derived from Bc5 bind fibronectin with a binding domain sharing identical amino acid residues including the conserved NNINNIY sequence. Furthermore, an interaction of *M. catarrhalis* UspA1/A2 with epithelial cells is via cell-associated fibronectin. The definition of these fibronectin binding domains is therefore an important step forward in the development of a vaccine against *M. catarrhalis*.

Interaction between *M. catarrhalis* and laminin

M. catarrhalis is a common cause of infectious exacerbations in patients with COPD. The success of this species in patients with COPD is probably related in part to

its large repertoire of adhesins. In addition, there are pathological changes such as loss of epithelial integrity with exposure of basement membrane where the laminin layer itself is thickened in smokers.[4] Some pathogens have been shown to be able to bind to laminin and thus may contribute to their ability to adhere to such damaged and denuded mucosal surfaces. These include pathogens known to cause significant disease in the airways such as *S. aureus* and *P. aeruginosa* amongst others.[7, 63]

We recently showed that both UspA1 and A2 bind fibronectin.[78] The fibronectin binding domains were located within $UspA1^{299-452}$ and $UspA2^{165-318}$. In this study, the N-terminal halves $UspA1^{50-491}$ and $UspA2^{30-351}$ (containing the fibronectin domains) also bound laminin. However, the smallest fragments that bound fibronectin, $UspA1^{299-452}$ and $UspA2^{165-318}$ did not bind laminin to any appreciable extent. In fact, fragments smaller than the N-terminal half of UspA1 ($UspA1^{50-491}$) losses all its laminin binding ability whereas with UspA2, only $UspA2^{30-170}$ bound laminin albeit at a lower level then the whole recombinant protein ($UspA2^{30-539}$). These findings suggest that perhaps different parts of the molecules might have different functional roles.

Comparing the smallest laminin binding regions of UspA1 and A2, we find that there is, however, little similarity by way of amino acid homology between $UspA2^{30-170}$ and $UspA1^{50-491}$ (data not shown). This is not surprising as it is a known fact that both proteins have a 'lollipop'-shaped globular head structure despite having only 22% identity in both N-terminal halves.[2, 32] We postulate that a tertiary structure is likely responsible for the interactions with laminin in the head region in vivo. The localization of the binding domains at the N-terminal end would be logical as this would be most exposed and in contact with the human basement membrane in vivo.

Bacterial factors mediating adherence to tissue and extracellular matrix (ECM) components are grouped together in a single family named "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMS). Since UspA1/A2 bind both fibronectin and laminin, these proteins can be designated MSCRAMMS. Our results suggest that UspA1 and A2 are multifunctional adhesins with different domains interacting with different ligands in the respiratory tract. Similar broad-spectrum binding profiles have been reported for other bacterial proteins such as YadA of *Yersinia enterocolitica* for which UspA1 and A2 bear a structural relationship. [45, 70] YadA too binds both fibronectin and laminin. [32]

In summary we have shown that UspA1/A2 are crucial to *M. catarrhalis* interaction with the basement membrane glycoprotein laminin and this will play an important role in the pathogenesis of infections in patients with COPD. [74]

Interaction between *M. catarrhalis* and C3 and C3met

Complement resistance is one of the most important bacterial virulence factors.[66] The majority (89 %) of *M. catarrhalis* isolates from patients with lower respiratory tract infections are resistant to complement-mediated killing.[34] *M. catarrhalis* UspA1 and A2 are crucial for bacterial survival in human serum in vivo [1, 15], and we have shown that these two outer membrane proteins bind to the complement fluid phase regulator of the classical pathway, C4BP.[58] In the present study, we demonstrate that *M. catarrhalis* can inhibit the alternative pathway by non-covalently binding of C3 (Figs. 17 and 18). The binding of C3 most likely also inhibits the classical pathway. This could, however, not be analysed in detail since *M. catarrhalis* also binds C4BP. Interestingly, the *M. catarrhalis*-dependent C3-binding is unique as several related moraxella subspecies as well as common human pathogenic bacteria do not bind C3 (Table 9). The

interactions with C3 and methylamine-treated C3 are mediated mainly by UspA2, whereas UspA1 has a minor role (Figs. 15 and 16). The C3-binding region of UspA2 was localized between the amino acid residues 200 to 458. This region contains a stretch of 140 amino acid residues that is 93 % identical to a region in UspA1.[2] However, despite this sequence similarity, UspA1 binds C3 to a much lower extent. This might be due to a specific difference in conformation between the proteins. The discrepancy in the C3 binding of UspA1 and UspA2 stands in contrast to the UspA1/A2 interaction with C4BP.[58]

M. catarrhalis is equally resistant to both the classical and alternative pathways (Fig. 12*B*). The bacterium binds C4BP that inhibits the classical pathway [58] and in this paper we demonstrate an interaction with the alternative pathway through binding of C3. To determine which of these mechanisms that is of most importance for the *M. catarrhalis* serum resistance in various in vivo situations is difficult. For example, the importance of the classical pathway will strongly depend on history of infections with *M. catarrhalis* and ability to generate complement-activating antibodies. However, every mechanism providing protection from the complement is certainly beneficial for a pathogen. Since C3 is a key molecule in the complement system, the binding of C3 most likely results in regulation of all three activation pathways and may contribute the most to serum resistance.

The importance of the complement system as a primary defence mechanism is mirrored by the fact that microbes have developed various strategies to interfere with and/ or neutralize components of the complement system.[42, 35, 88] In addition to *M. catarrhalis, S. pyogenes, Bordetella pertussis, E. coli* K1, *Candida albicans*, and *N. gonorrhoeae* express specific surface molecules that bind C4BP and as a consequence protect the bacteria against the classical

complement pathway.[8, 9, 52, 58, 64, 65, 80] In addition to inhibition of the classical pathway, several bacteria (e.g., *C. albicans, N. meningitides, S. pyogenes,* and *S. pneumoniae;* for reviews see [68, 89] bind factor H and factor H-like molecule and hence are partially protected against the alternative complement pathway.

UspA1 and A2 absorb C3 from serum and hereby most likely inhibit the complement activation. Similarly, the Pneumococcal Surface Protein A (PspA) appears to inhibit the alternative pathway both in vitro and in vivo. PspA is an important virulence factor for *S. pneumoniae*. PspA-deficient pneumococcal strains are readily cleared from the blood, whereas the PspA-expressing strains survive.[82] Furthermore, in a murine model of bacteremia, PspA-deficient pneumococci have a significantly reduced virulence compared with pneumococci that express PspA.[11] It has been demonstrated that more C3b is deposited on PspA-negative pneumococci than on PspA-positive.[67, 82] Thus, expression of PspA reduces the complement-mediated clearance and phagocytosis of *S. pneumoniae* by limiting opsonization by C3b.[12, 67] PspA-deficient pneumococci that are not virulent in normal mice become virulent in C3-deficient and factor B-deficient mice.[82]

To our knowledge, there are only two examples of bacterial proteins that non-covalently bind C3 and thereby interfere with complement function. The first one is the extracellular fibrinogen-binding protein (Efb) of *Staphylococcus aureus*, which was found to bind C3b.[44] Efb inhibits both the classical and alternative pathways independently of the thioester conformation, i.e., the binding to C3b is non-covalent. The second example is the pneumococcal choline-binding protein (CbpA), which has been shown to bind methylamine-treated C3, suggesting a non-covalent interaction that is not dependent on complement activation.[16] CbpA is a component of the pneumococcal cell

wall, but may only bind C3 when the CbpA is secreted. In order to test this hypothesis, which is not firmly established in the literature, we analyzed eleven different pneumococcal isolates for C3 binding (methylamine-treated C3 or NHS-EDTA) by flow cytometry (Fig. 12*B* and Table 9). No bound C3 could be detected on the surface of *S. pneumoniae*. When lysates of *S. pneumoniae* and culture supernatants were analyzed on Western blots using methylamine-treated C3 followed by an anti-human C3 pAb, we confirmed the results by Cheng and collaborators [16] (not shown). In the light of Efb and CbpA, which both are C3-binding proteins secreted by two Gram-positive bacteria, the Gram-negative *M. catarrhalis* is a unique species with membrane anchored proteins that bind C3 and inhibit the alternative pathway at the surface of the bacterium.

The yeast *Candida albicans* has been shown to bind C3b, iC3b and C3d. However, C3b is bound at a considerably lower affinity than iC3b and C3d.[29] We found a large difference between C3 binding to *M. catarrhalis* and *C. albicans* (not shown); despite that candida bound C3met (56 % positive cells), the mean fluorescence intensity (mfi) was only < 2.0 as compared to mfi 36.9 for *M. catarrhalis*. Furthermore, no detectable binding was seen when *C. albicans* was incubated with EDTA-treated serum. Two C3d-binding proteins have been isolated from *C. albicans* and the most characterized protein is a 60 kDa mannoprotein that initially was recognized by an antibody directed against human complement receptor 2 (CD21).[13] However, *M. catarrhalis* UspA1 and A2 were not recognized by a polyclonal antibody directed against CD21 (not shown). In parallel with staphylococci and pneumococci [52, 64], a secreted C3d-binding protein from *C. albicans* also exists.[72] Finally, a *C. albicans* iC3b receptor has been isolated and is structurally similar to human CR3 (CD11b).[3] The mechanisms by which these receptors may participate in pathogenesis are not fully known.

The above examples of C3 binding pathogens are notably different from *M. catarrhalis* in that these species often are blood stream isolates. *M. catarrhalis* is mucosal pathogen with rare instances of bacteremic infections. Hence, the binding and inactivating C3 most likely occur at the mucosal surface. This is supported by the fact that there is strong ongoing complement activation and consequent inflammation in disease state such as acute otitis media.[57] The complement proteins are believed to be transported to the mucosal surface due to exudation of plasma.[26, 62] In middle-ear effusions (MEEs) from children for example, strongly elevated concentrations of C3 products can also be found.[51] In addition, complement factors in MEEs fluid have been shown to be important in the bactericidal activity against other mucosal agents such as non-typable *H. influenzae*.[75] *M. catarrhalis* is a strict human pathogen. It does not cause diseases such as otitis media or pneumonia in animals. A mouse pulmonary clearance model and an otitis media model with chinchilla has been used at several occasions. However, neither otitis media nor pneumonia develops and bacteria are rapidly cleared .[19, 83] It is thus difficult to test the biological significance of bacterial C3 binding in vivo. Since UspA1 and A2 are multifunctional proteins [1, 15, 31, 43, 58, 78], it would be impossible to relate any differences in the clearance of *M. catarrhalis* to C3 binding. In particular the fact that UspA1 is an important adhesin of *M. catarrhalis* and binds both CEACAM1 and fibronectin [31, 78] would most likely affect the clearance. Nevertheless, due to the strong complement activation in disease states such as otitis media, moraxella-dependent binding of C3 may represent an important way of combating the mucosal defense.

The fact that *M. catarrhalis* hampers the human immune system in several ways might explain why *M. catarrhalis* is such a common inhabitant of the respiratory tract [73]. In

conclusion, *M. catarrhalis* has developed sophisticated ways of combating both the humoral and innate immune systems. The present data show that *M. catarrhalis* has a unique C3-binding capacity at the bacterial cell surface that cannot be found in other bacterial species.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Peptide consisting of Sequence ID No. 2, or a fragment, homologue, functional equivalent, or hydroxylation, sulphonation or glycosylation product thereof which retain fibronectin binding properties.

2. Peptide consisting of Sequence ID No. 3, or a fragment, homologue, functional equivalent, or hydroxylation, sulphonation or glycosylation product thereof which retain fibronectin binding properties.

3. Use of at least one peptide according to any one of claims 1 to 2 for the production of a medicament for the treatment of prophylaxis of an infection.

4. Use according to claim 3, wherein the infection is caused by *Moraxella catarrhalis*.

5. Use according to claim 3 or 4, for the prophylaxis or treatment of otitis media, sinusitis or lower respiratory tract infections.

6. A ligand comprising a fibronectin binding domain, said ligand consisting of an amino acid sequence selected from the group consisting of Sequence ID No. 2 or Sequence ID No. 3, or a fragment, homologue, functional equivalent, or hydroxylation, sulphonation or glycosylation product thereof which retain fibronectin binding properties.

7. A fusion protein comprising one or more ligands according to claim 6.

8. A medicament comprising one or more ligands according to claim 6 or 7 and one or more pharmaceutically acceptable adjuvants, vehicles, excipients, binders, carriers, or preservatives.

9. A vaccine comprising one or more ligands according to claim 6 or 7 and one or more pharmaceutically acceptable

adjuvants, vehicles, excipients, binders, carriers, or preservatives.

10. A method of treating or preventing an infection in an individual comprising administering a pharmaceutically effective amount of a medicament or a vaccine according to claim 8 or 9.

11. A method according to claim 10, wherein the infection is caused by *Moraxella catarrhalis*.

12. A nucleic acid sequence encoding a ligand, protein or peptide of the present invention, as well as homologues, polymorphisms, fragments, and splice variants thereof.

13. A polypeptide or a polypeptide truncate consisting of at least one of the conserved sequences of Sequence ID No. 2 or Sequence ID No. 3 or a fragment, homologue, functional equivalent, or hydroxylation, sulphonation or glycosylation product thereof with the ability of binding fibronectin.

14. A peptide according to claim 1 or claim 2, or use according to any one of claims 3 to 5, or a ligand according to claim 6, or a fusion protein according to claim 7, or a medicament according to claim 8, or a vaccine according to claim 9, or a method according to claim 10 or claim 11, or a nucleic acid sequence according to claim 12, or a polypeptide or polypeptide truncate according to claim 13 substantially as hereinbefore defined.

Figure 1

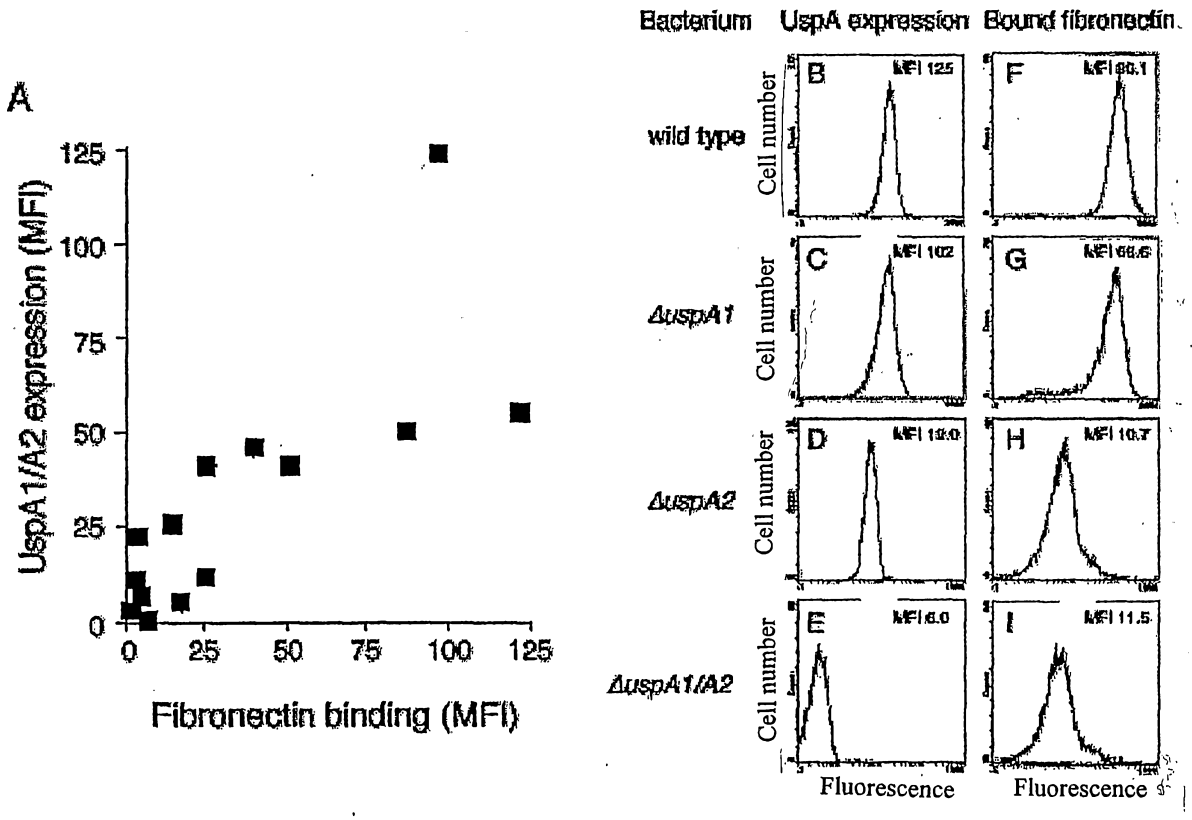

A
UspA1/A2 expression (MFI)
125
100
75
50
25
0
0
25
50
75
100
125
Fibronectin binding (MFI)
Bacterium
UspA expression
Bound fibronectin
wild type
ΔuspA1
ΔuspA2
ΔuspA1/A2
Cell number
B
MFI 125
F
C
MFI 102
G
D
MFI 13.0
H
MFI 10.7
E
MFI 6.0
I
MFI 11.5
Fluorescence
Fluorescence

Figure 2

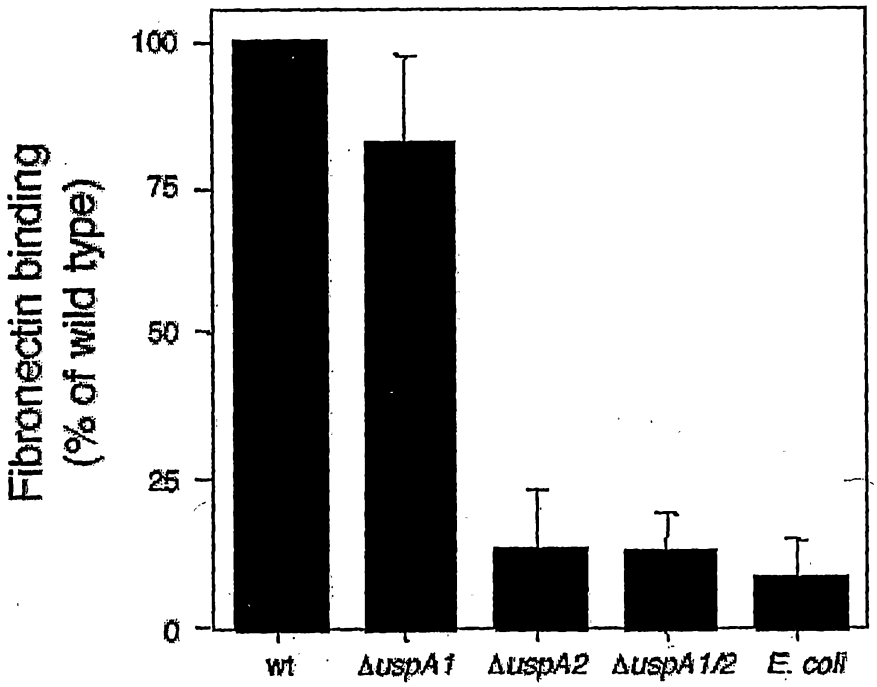

Fibronectin binding
(% of wild type)
100
75
50
25
0
wt
ΔuspA1
ΔuspA2
ΔuspA1/2
E. coli

Figure 3

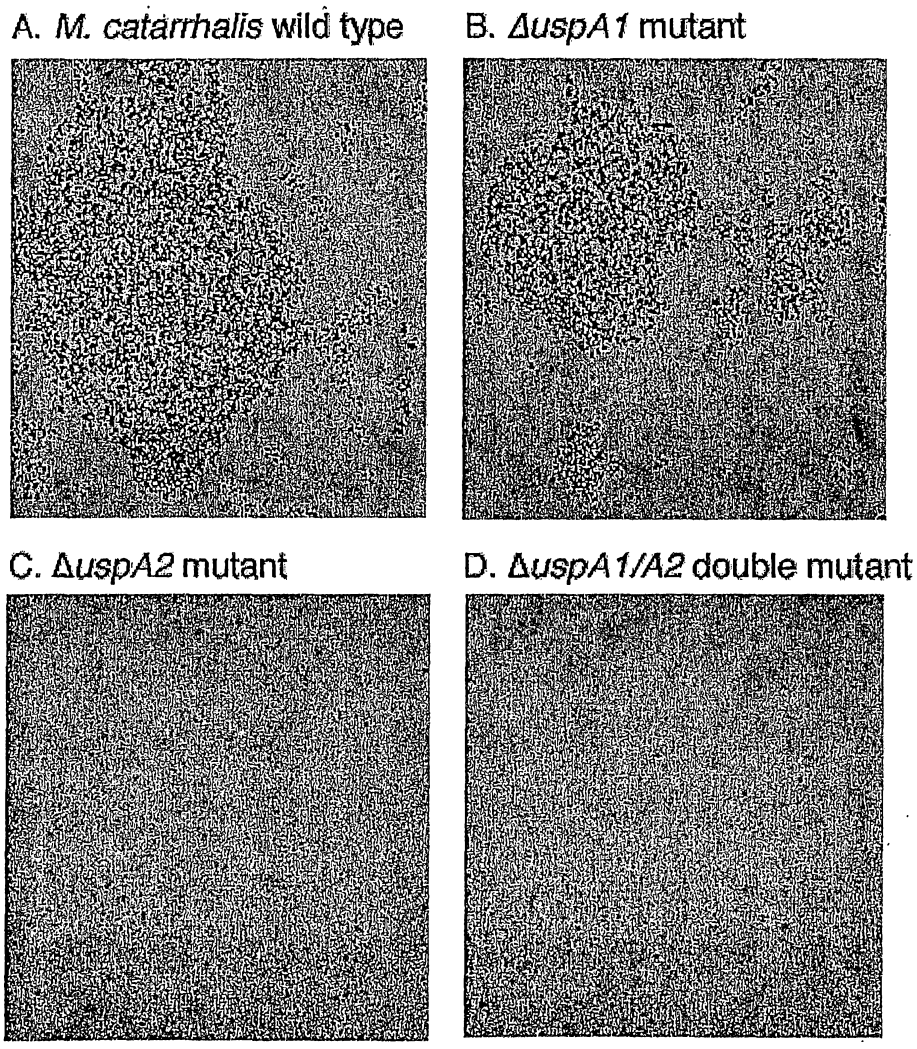

A. *M. catarrhalis* wild type
B. *ΔuspA1* mutant
C. Δ*uspA2* mutant
D. Δ*uspA1/A2* double mutant

Figure 4

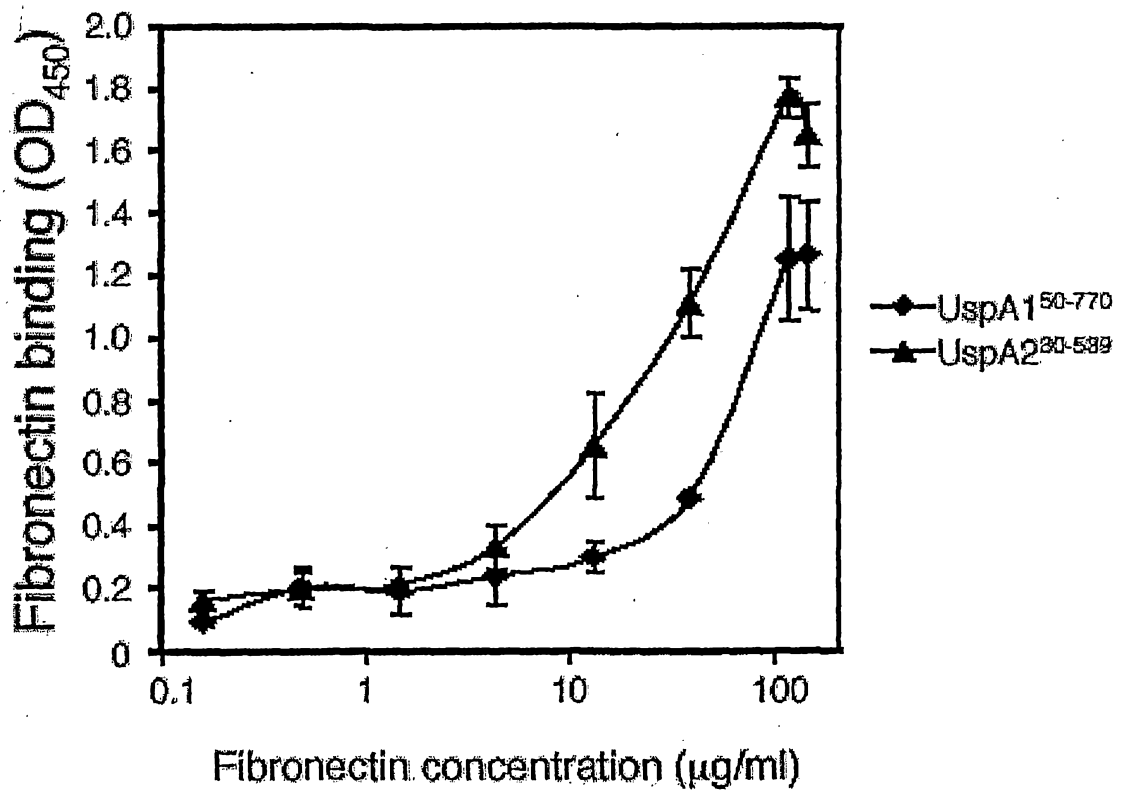
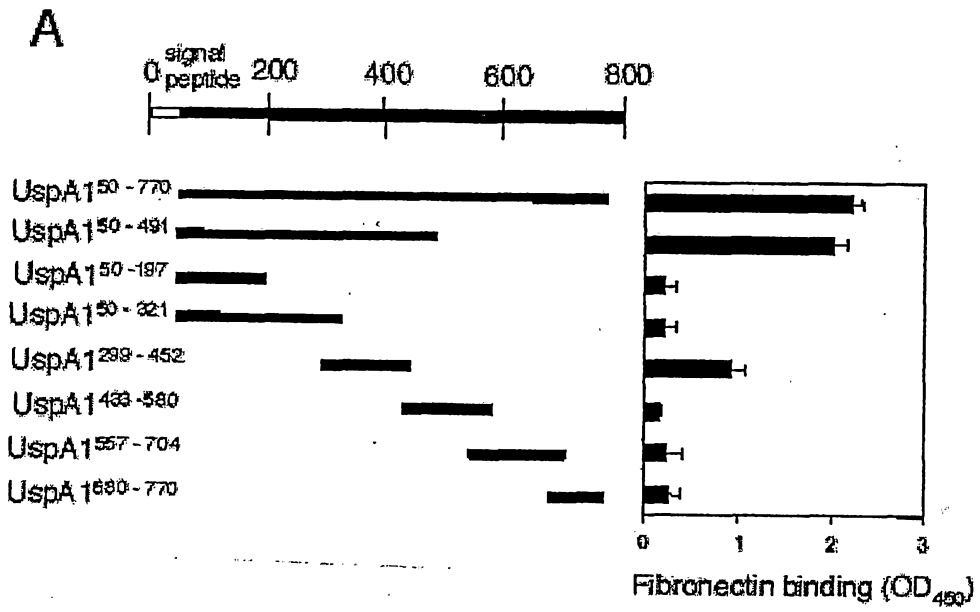

Fibronectin binding (OD_{450})
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0.1
1
10
100
Fibronectin concentration (µg/ml)
UspA1$^{50-770}$
UspA2$^{30-589}$

Figure 5


A
0
signal peptide
200
400
600
800
UspA1 50 - 770
UspA1 50 - 491
UspA1 50 - 197
UspA1 50 - 321
UspA1 239 - 452
UspA1 433 - 580
UspA1 557 - 704
UspA1 680 - 770
0
1
2
3
Fibronectin binding (OD450)

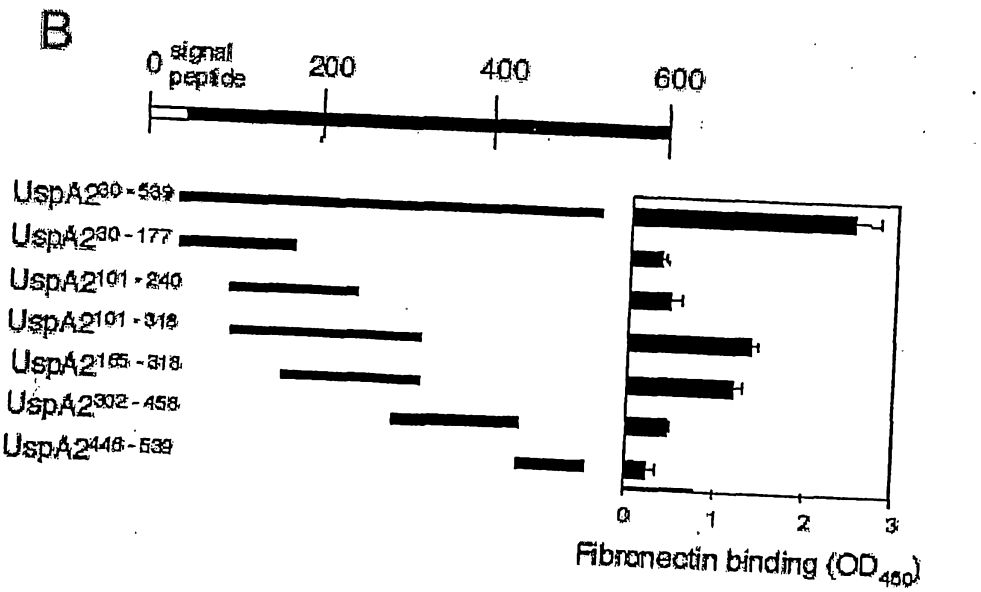

B
0
signal peptide
200
400
600
UspA2 30 - 539
UspA2 30 - 177
UspA2 101 - 240
UspA2 101 - 318
UspA2 165 - 318
UspA2 302 - 458
UspA2 446 - 539
0
1
2
3
Fibronectin binding (OD450)

Figure 6

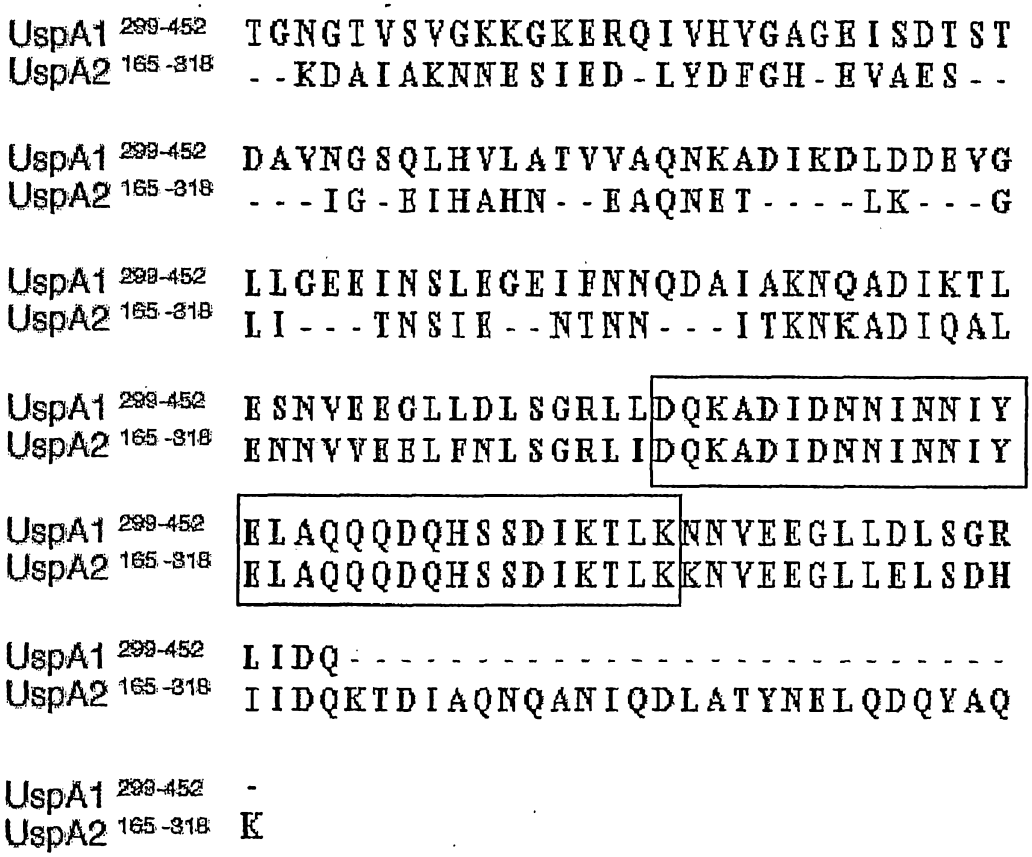

UspA1 299-452 TGNGTVSVGKKGKERQIVHVGAGEISDTST
UspA2 165-318 --KDAIAKNNESIED-LYDFGH-EVAES--
UspA1 299-452 DAVNGSQLHVLATVVAQNKADIKDLDDEVG
UspA2 165-318 ---IG-EIHAHN--EAQNET----LK---G
UspA1 299-452 LLGEEINSLEGEIFNNQDAIAKNQADIKTL
UspA2 165-318 LI---TNSIE--NTNN---ITKNKADIQAL
UspA1 299-452 ESNVEEGLLDLSGRLLDQKADIDNNINNIY
UspA2 165-318 ENNVVEELFNLSGRLIDQKADIDNNINNIY
UspA1 299-452 ELAQQQDQHSSDIKTLKNNVEEGLLDLSGR
UspA2 165-318 ELAQQQDQHSSDIKTLKKNVEEGLLELSDH
UspA1 299-452 LIDQ----------
UspA2 165-318 IIDQKTDIAQNQANIQDLATYNELQDQYAQ
UspA1 299-452 -
UspA2 165-318 K

Figure 7

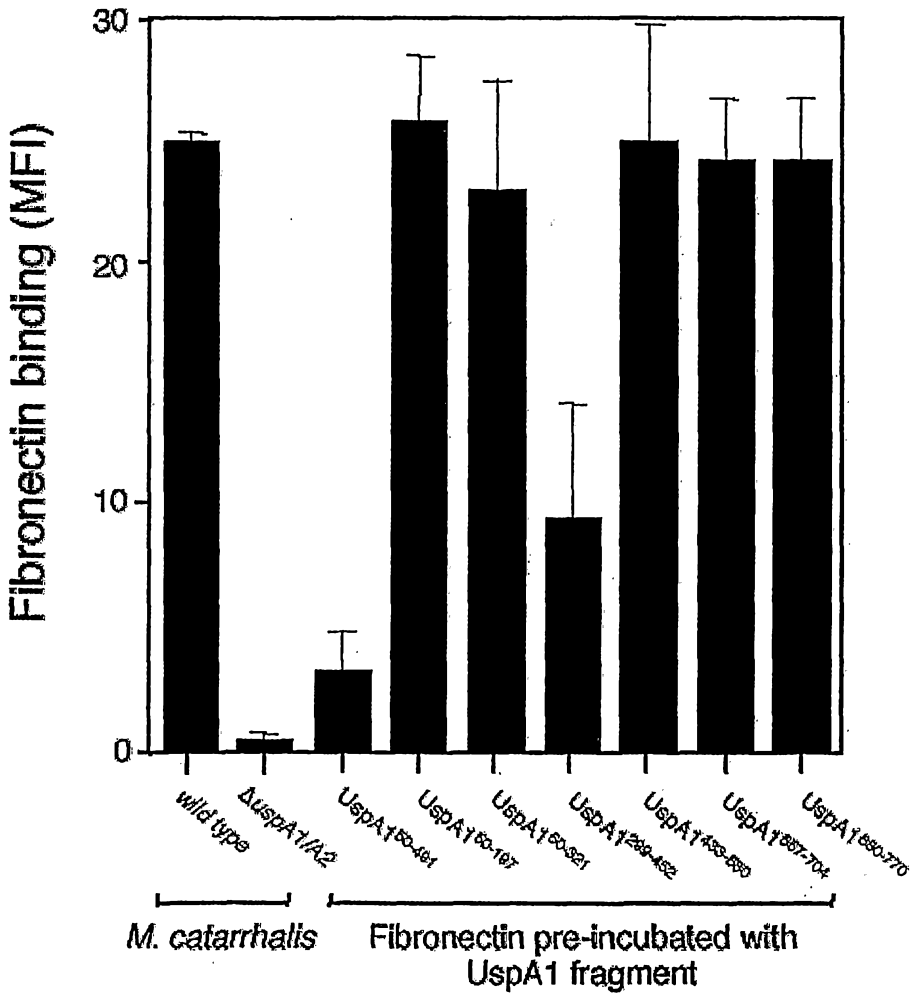
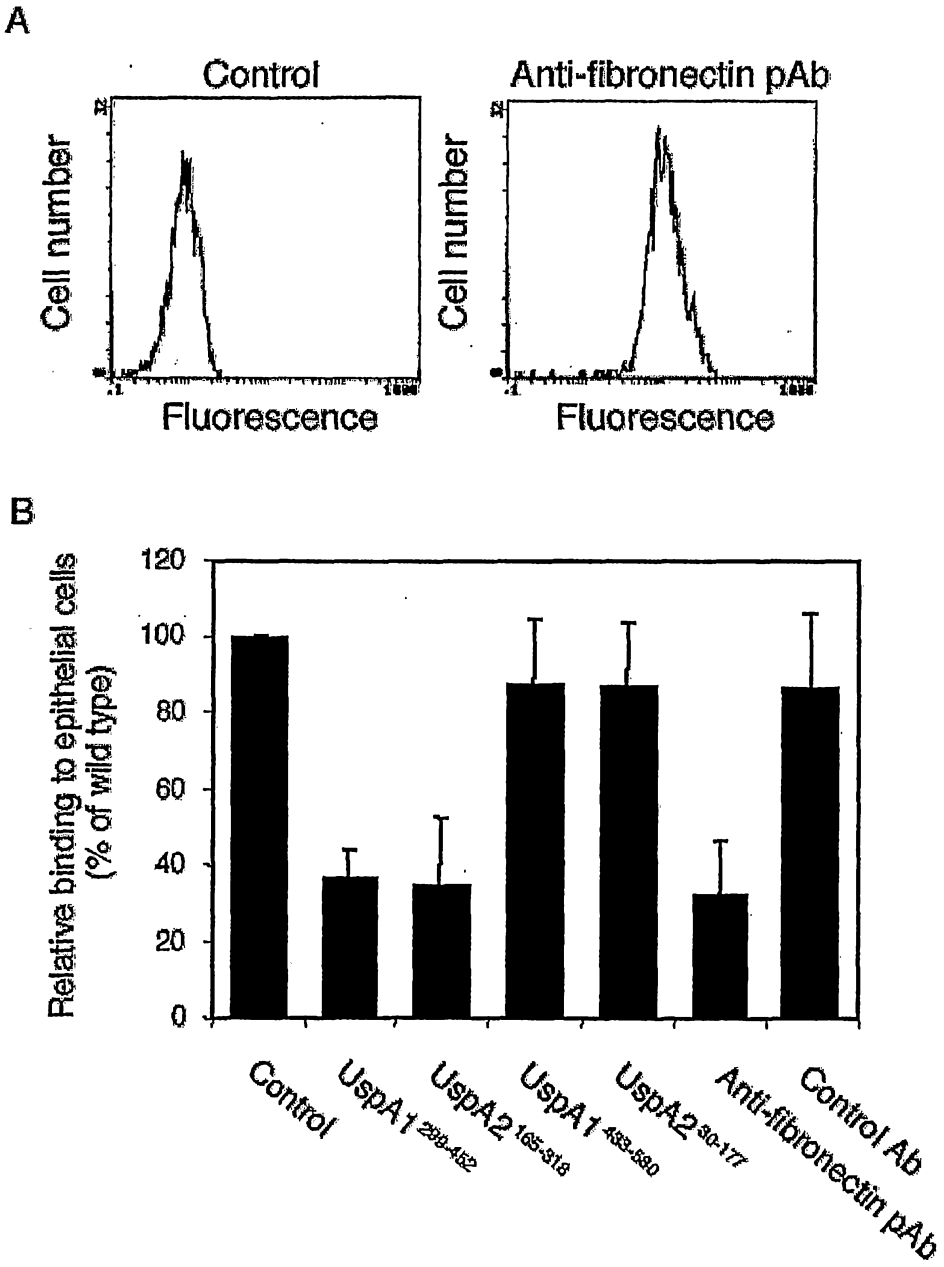

Fibronectin binding (MFI)
30
20
10
0
wild type
ΔuspA1/A2
UspA1150-481
UspA1150-187
UspA1150-521
UspA1299-452
UspA1455-693
UspA1687-704
UspA1690-770
M. catarrhalis
Fibronectin pre-incubated with UspA1 fragment

Figure 8


A
Control
Anti-fibronectin pAb
Cell number
Fluorescence
B
Relative binding to epithelial cells (% of wild type)
120
100
80
60
40
20
0
Control
UspA1 239-452
UspA2 165-318
UspA1 433-580
UspA2 30-177
Anti-fibronectin pAb
Control Ab

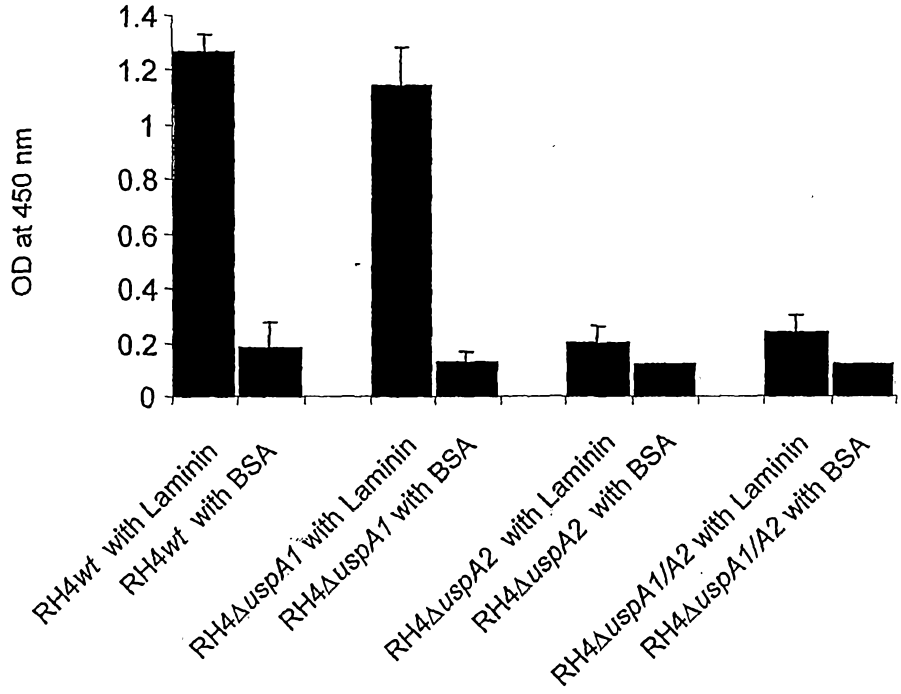


Figure 9*A*.

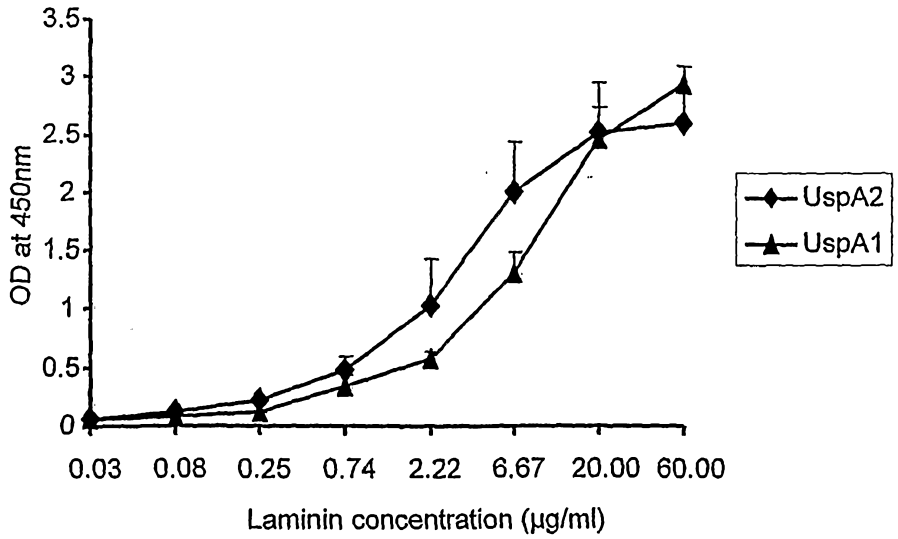


Figure 9*B*.

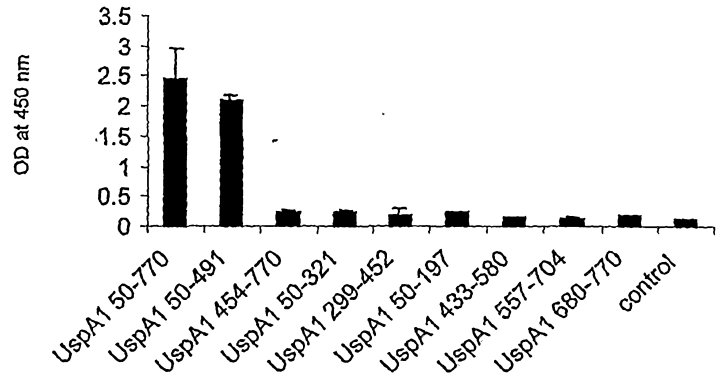


Figure 10*A*.

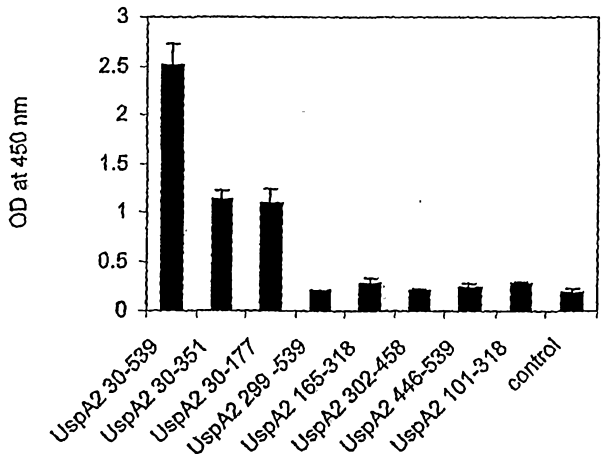


Figure 10*B*.

Figure 11

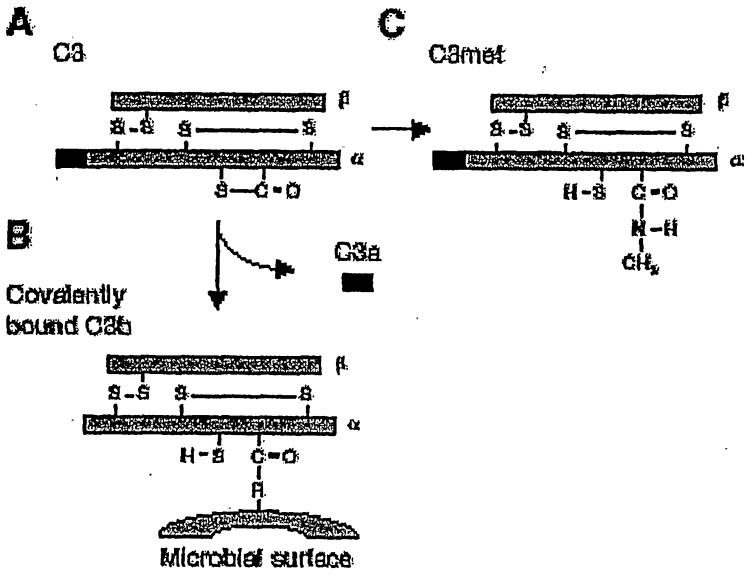


Figure 12

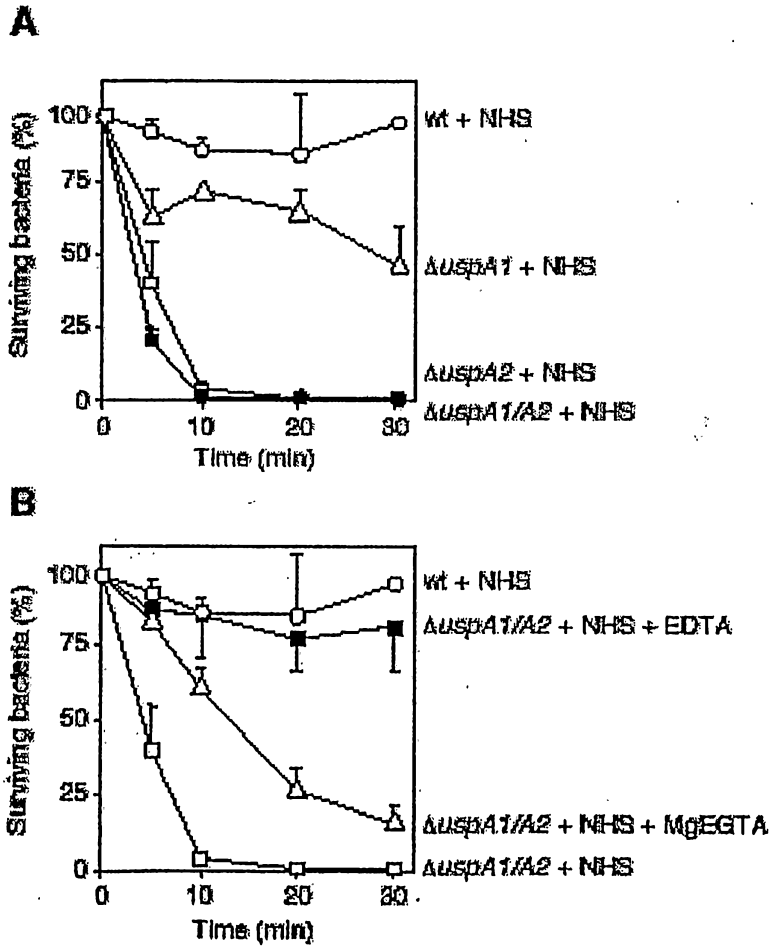

A
Surviving bacteria (%)
100
75
50
25
0
0
10
20
30
Time (min)
wt + NHS
ΔuspA1 + NHS
ΔuspA2 + NHS
ΔuspA1/A2 + NHS
B
Surviving bacteria (%)
100
75
50
25
0
0
10
20
30
Time (min)
wt + NHS
ΔuspA1/A2 + NHS + EDTA
ΔuspA1/A2 + NHS + MgEGTA
ΔuspA1/A2 + NHS

Figure 13

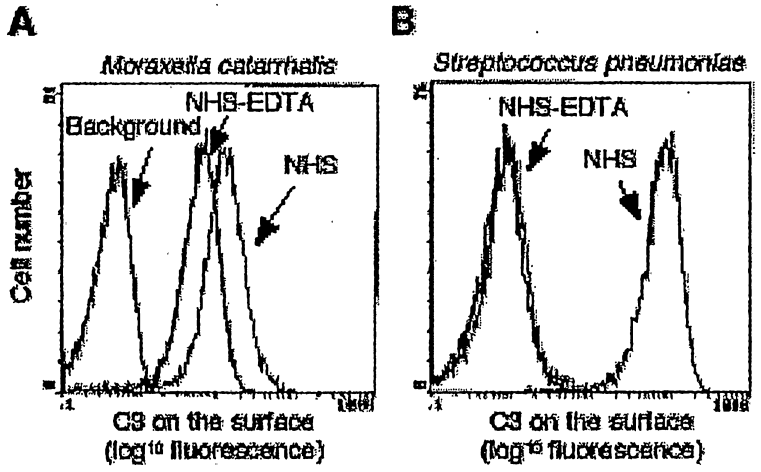

A
Moraxella catarrhalis
NHS-EDTA
Background
NHS
Cell number
C3 on the surface
(log10 fluorescence)
B
Streptococcus pneumoniae
NHS-EDTA
NHS
C3 on the surface
(log10 fluorescence)

Figure 14

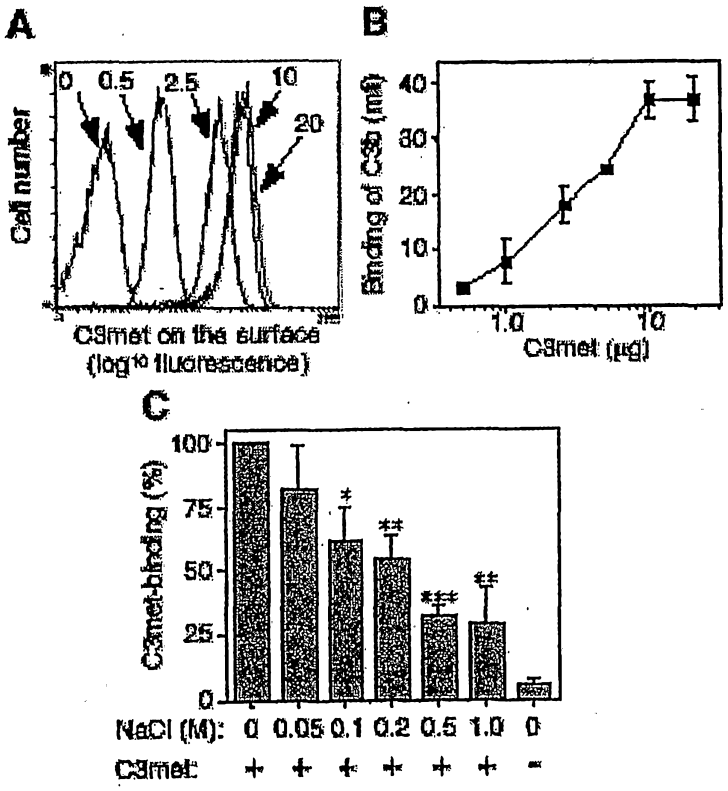

A
B
C
Cell number
0
0.5
2.5
10
20
C3met on the surface
(log10 fluorescence)
Binding of C3b (nM)
40
30
20
10
0
1.0
10
C3met (µg)
C3met-binding (%)
100
75
50
25
0
NaCl (M): 0 0.05 0.1 0.2 0.5 1.0 0
C3met: + + + + + + -

Figure 15

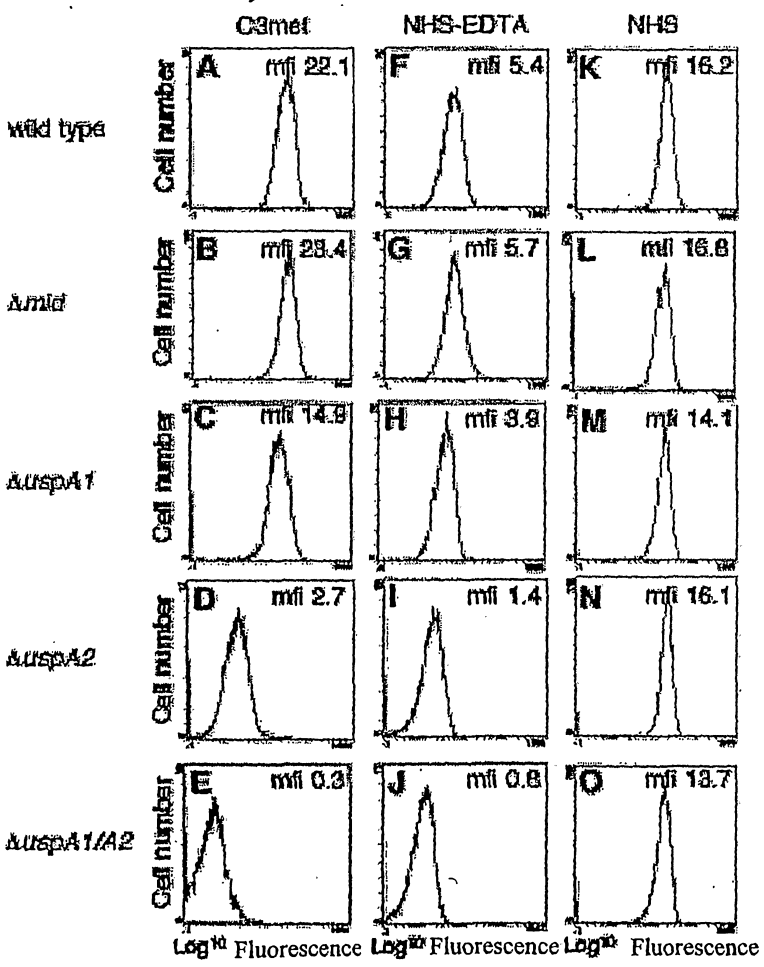

C3met
NHS-EDTA
NHS
wild type
Δmid
ΔuspA1
ΔuspA2
ΔuspA1/A2
Cell number
A mfi 22.1
F mfi 5.4
K mfi 16.2
B mfi 28.4
G mfi 5.7
L mfi 16.8
C mfi 14.9
H mfi 3.9
M mfi 14.1
D mfi 2.7
I mfi 1.4
N mfi 16.1
E mfi 0.3
J mfi 0.8
O mfi 13.7
Log Fluorescence
Log Fluorescence
Log Fluorescence

Figure 16

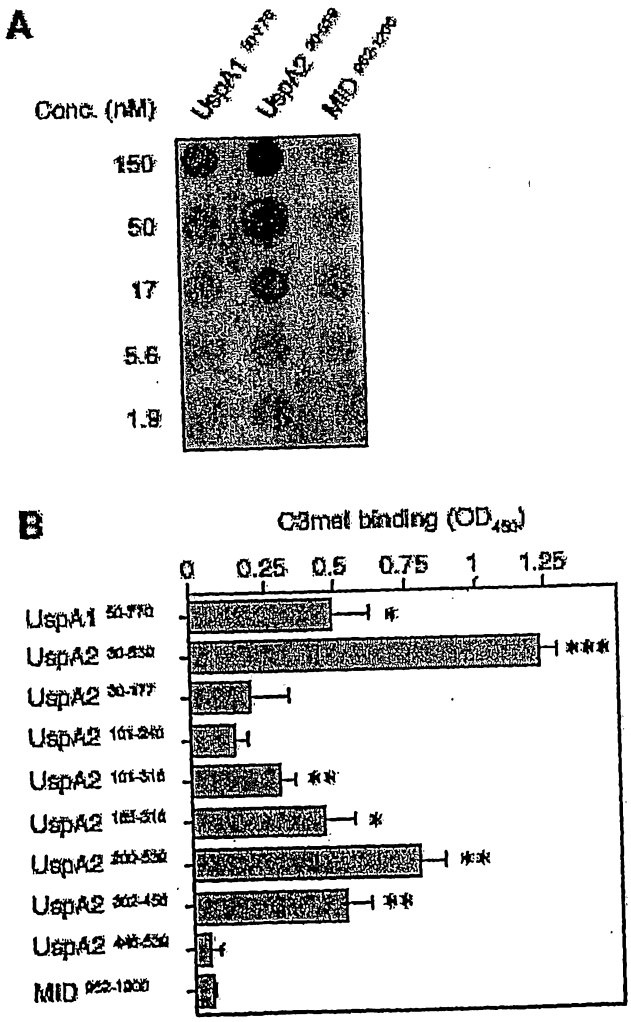

A
Conc. (nM)
UspA1
UspA2
MID
150
50
17
5.6
1.9
B
C3mat binding (OD450)
0
0.25
0.5
0.75
1
1.25
UspA1
UspA2
UspA2
UspA2
UspA2
UspA2
UspA2
UspA2
MID

Figure 17

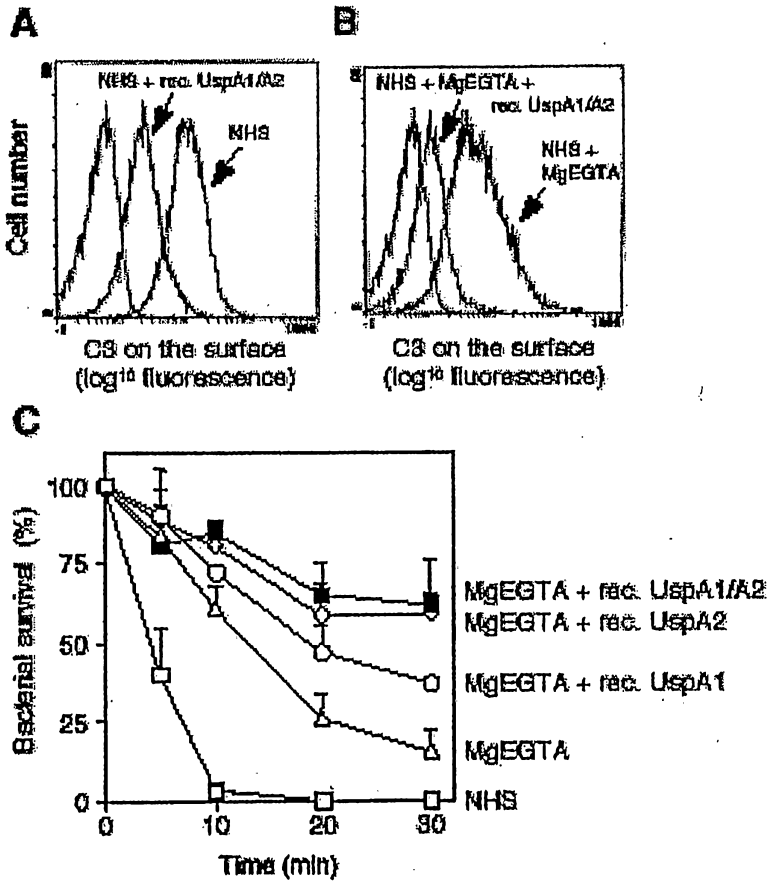

A
B
NHS + rec. UspA1/A2
NHS
Cell number
C3 on the surface
(log10 fluorescence)
NHS + MgEGTA +
rec. UspA1/A2
NHS +
MgEGTA
C3 on the surface
(log10 fluorescence)
C
Bacterial survival (%)
100
75
50
25
0
0
10
20
30
Time (min)
MgEGTA + rec. UspA1/A2
MgEGTA + rec. UspA2
MgEGTA + rec. UspA1
MgEGTA
NHS

Figure 18

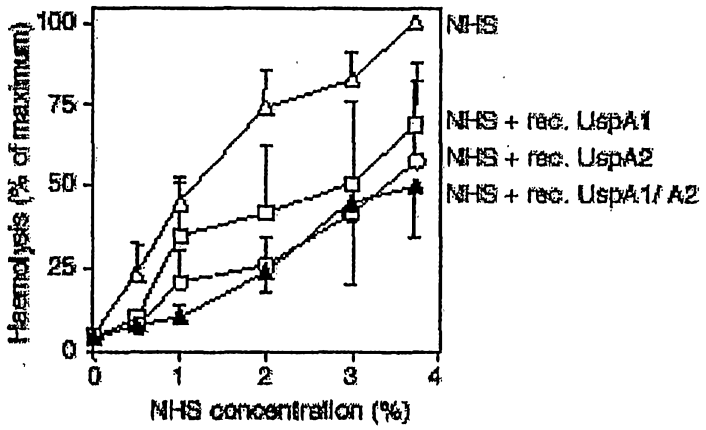
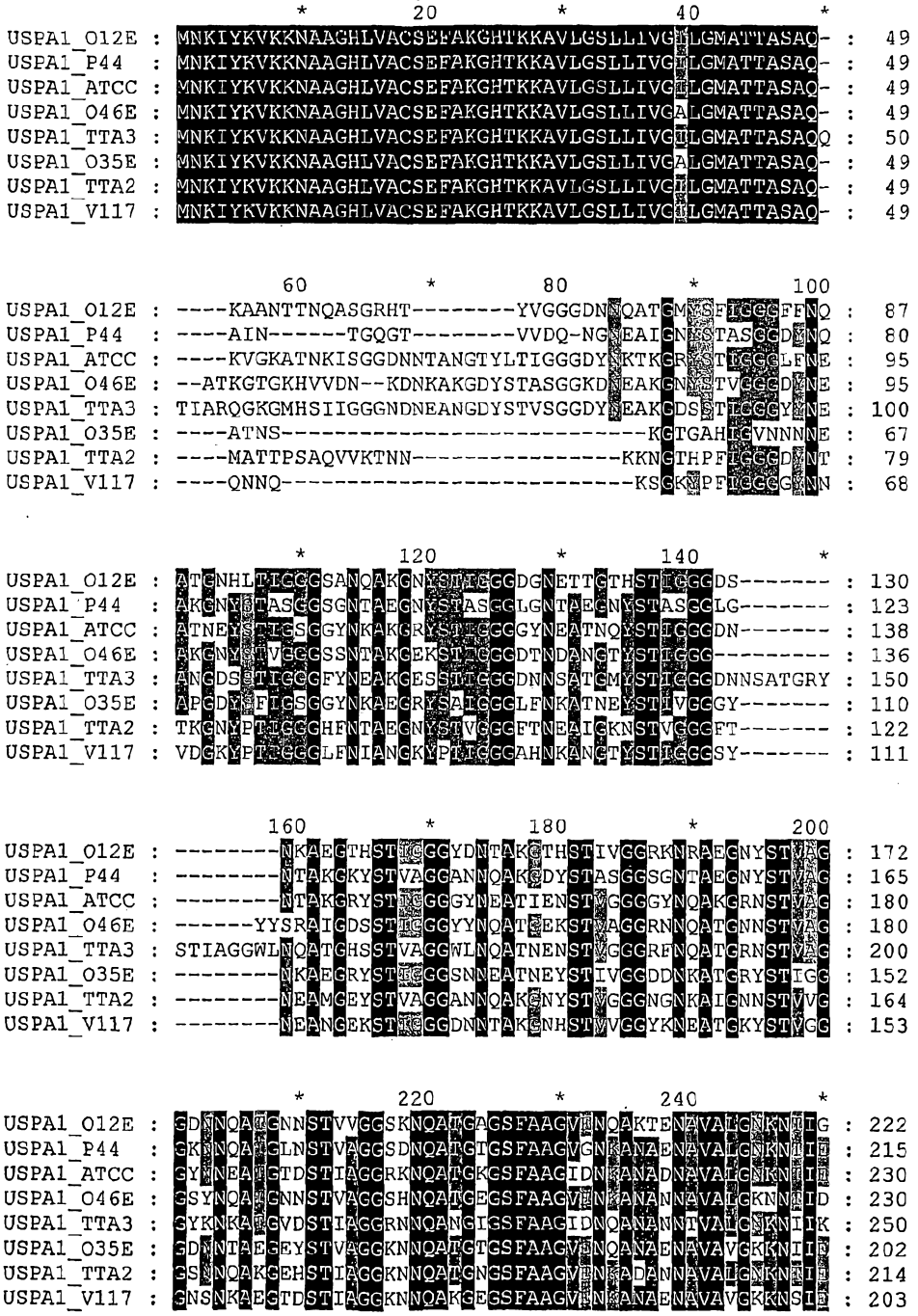
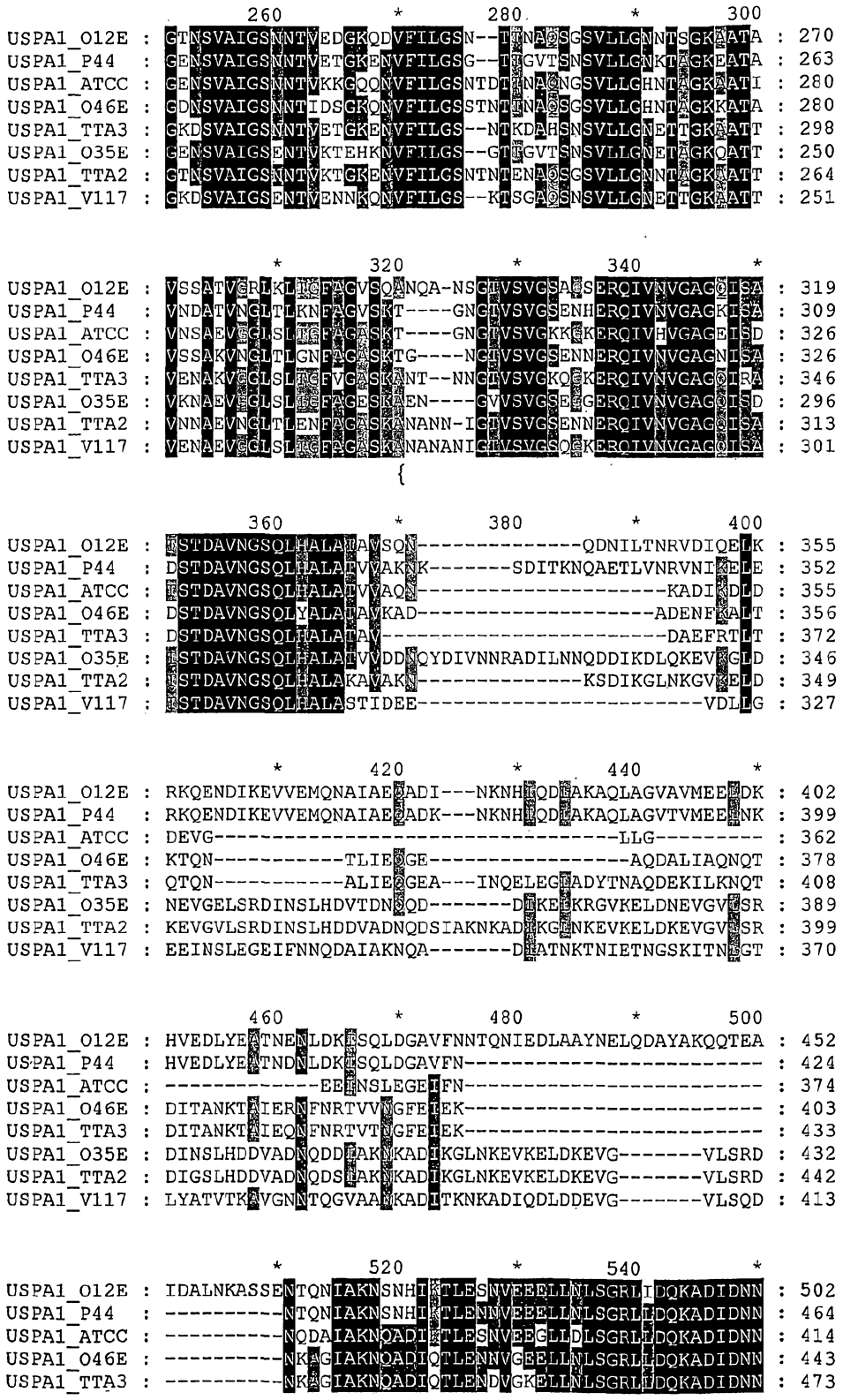

Haemolysis (% of maximum)
100
75
50
25
0
0
1
2
3
4
NHS concentration (%)
NHS
NHS + rec. UspA1
NHS + rec. UspA2
NHS + rec. UspA1/ A2

Fig. 19

Pileup – beginning and end of the fibronectin binding domain marked with { and }




260 * 280 * 300
USPA1_O12E : GTNSVAIGSNNTVEDGKQDVFILGSN--TTNAQSGSVLLGNNTSGKAATA : 270
USPA1_P44 : GENSVAIGSNNTVETGKENVFILGSG--TTGVTSNSVLLGNKTAGKEATA : 263
USPA1_ATCC : GENSVAIGSNNTVKKGQQNVFILGSNTDTTNAQNGSVLLGHNTAGKAATI : 280
USPA1_O46E : GDNSVAIGSNNTIDSGKQNVFILGSSTNTTNAQSGSVLLGHNTAGKKATA : 280
USPA1_TTA3 : GKDSVAIGSNNTVETGKENVFILGS--NTKDAHSNSVLLGNETTGKAATT : 298
USPA1_O35E : GENSVAIGSENTVKTEHKNVFILGS--GTTGVTSNSVLLGNETAGKQATT : 250
USPA1_TTA2 : GTNSVAIGSNNTVKTGKENVFILGSNTNTENAQSGSVLLGNNTAGKAATT : 264
USPA1_V117 : GKDSVAIGSENTVENNKQNVFILGS--KTSGAQSNSVLLGNETTGKAATT : 251
* 320 * 340 *
USPA1_O12E : VSSATVGRLKLTGFAGVSQANQA-NSGTVSVGSAQSERQIVNVGAGQISA : 319
USPA1_P44 : VNDATVNGLTLKNFAGVSKT----GNGTVSVGSENHERQIVNVGAGKISA : 309
USPA1_ATCC : VNSAEVEGLSLTGFAGASKT----GNGTVSVGKKQKERQIVHVGAGEISD : 326
USPA1_O46E : VSSAKVNGLTLGNFAGASKTG----NGTVSVGSENNERQIVNVGAGNISA : 326
USPA1_TTA3 : VENAKVGGLSLTGFVGASKANT--NNGTVSVGKQQKERQIVNVGAGQIRA : 346
USPA1_O35E : VKNAEVEGLSLTGFAGESKAEN----GVVSVGSEQGERQIVNVGAGQISD : 296
USPA1_TTA2 : VNNAEVNGLTLENFAGASKANANN-IGTVSVGSENNERQIVNVGAGQISA : 313
USPA1_V117 : VENAEVGGLSLTGFAGASKANANANIGTVSVGSQQKERQIVNVGAGQISA : 301
{
360 * 380 * 400
USPA1_O12E : ISTDAVNGSQLHALAIAVSQN----------QDNILTNRVDIQELK : 355
USPA1_P44 : DSTDAVNGSQLHALAVVAKNK-------SDITKNQAETLVNRVNIKELE : 352
USPA1_ATCC : ISTDAVNGSQLHALAIVVAQN----------KADIKDLD : 355
USPA1_O46E : DSTDAVNGSQLYALAIAVKAD----------ADENFKALT : 356
USPA1_TTA3 : DSTDAVNGSQLHALAIAV----------DAEFRTLT : 372
USPA1_O35E : ISTDAVNGSQLHALAIVVDDQYDIVNNRADILNNQDDIKDLQKEVKGLD : 346
USPA1_TTA2 : ISTDAVNGSQLHALAKAVAKN----------KSDIKGLNKGVKELD : 349
USPA1_V117 : ISTDAVNGSQLHALASTIDEE----------VDLLG : 327
* 420 * 440 *
USPA1_O12E : RKQENDIKEVVEMQNAIAEQADI---NKNHIQDIAKAQLAGVAVMEEIDK : 402
USPA1_P44 : RKQENDIKEVVEMQNAIAEQADK---NKNHIQDIAKAQLAGVTVMEEINK : 399
USPA1_ATCC : DEVG----------LLG--------- : 362
USPA1_O46E : KTQN----------TLIEQGE----------AQDALIAQNQT : 378
USPA1_TTA3 : QTQN----------ALIEQGEA---INQELEGIADYTNAQDEKILKNQT : 408
USPA1_O35E : NEVGELSRDINSLHDVTDNQQD-------DIKEIKRGVKELDNEVGVVSR : 389
USPA1_TTA2 : KEVGVLSRDINSLHDDVADNQDSIAKNKADIKGLNKEVKELDKEVGVVSR : 399
USPA1_V117 : EEINSLEGEIFNNQDAIAKNQA-------DIATNKTNIETNGSKITNIGT : 370
460 * 480 * 500
USPA1_O12E : HVEDLYEATNENLDKISQLDGAVFNNTQNIEDLAAYNELQDAYAKQQTEA : 452
USPA1_P44 : HVEDLYEATNDNLDKISQLDGAVFN---------- : 424
USPA1_ATCC : ----------EEINSLEGEIFN---------- : 374
USPA1_O46E : DITANKTAIERNFNRTVVNGFEIEK---------- : 403
USPA1_TTA3 : DITANKTAIEQNFNRTVTNGFEIEK---------- : 433
USPA1_O35E : DINSLHDDVADNQDDIAKNKADIKGLNKEVKELDKEVG-------VLSRD : 432
USPA1_TTA2 : DIGSLHDDVADNQDSIAKNKADIKGLNKEVKELDKEVG-------VLSRD : 442
USPA1_V117 : LYATVTKAVGNNTQGVAANKADITKNKADIQDLDDEVG-------VLSQD : 413
* 520 * 540 *
USPA1_O12E : IDALNKASSENTQNIAKNSNHIKTLESNVEEELLNLSGRLIDQKADIDNN : 502
USPA1_P44 : ----------NTQNIAKNSNHIKTLENNVEEELLNLSGRLIDQKADIDNN : 464
USPA1_ATCC : ----------NQDAIAKNQADIKTLESNVEEGLLDLSGRLIDQKADIDNN : 414
USPA1_O46E : ----------NKAGIAKNQADIQTLENNVGEELLNLSGRLIDQKADIDNN : 443
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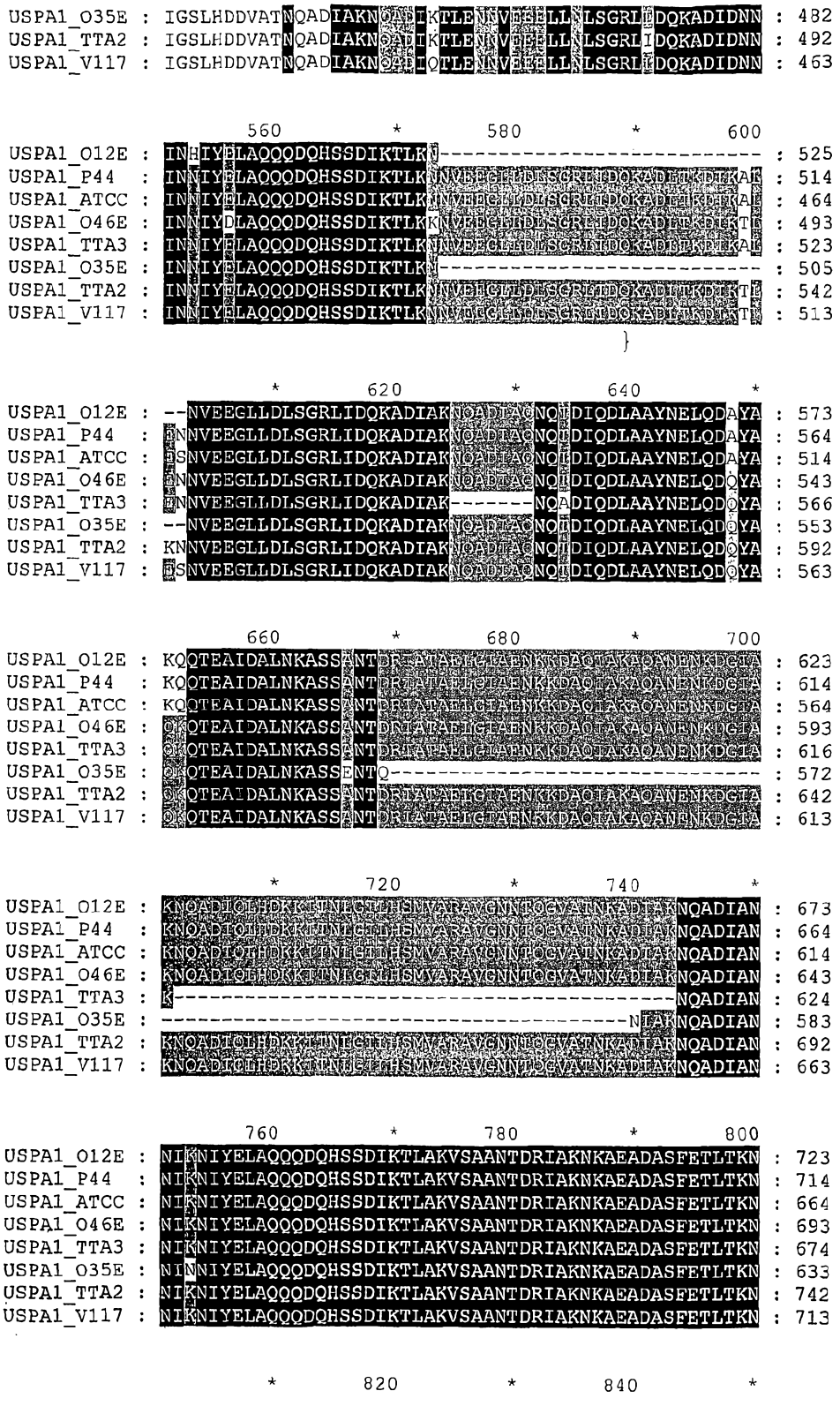
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USPA1_V117 : IGSLHDDVATNQADIAKNQADIQTLENNVTEFLLNLSGRLIDQKADIDNN : 463
560 * 580 * 600
USPA1_O12E : INHIYELAQQQDQHSSDIKTLKN---------- : 525
USPA1_P44 : INNIYELAQQQDQHSSDIKTLKNNVEEGLLDLSGRLIDQKADIDKDIKAL : 514
USPA1_ATCC : INNIYELAQQQDQHSSDIKTLKNNVEEGLLDLSGRLIDQKADIDKDIKAL : 464
USPA1_O46E : INNIYDLAQQQDQHSSDIKTLKKNVEDGLLDLSGRLIDQKADIDKDIKTL : 493
USPA1_TTA3 : INNIYELAQQQDQHSSDIKTLKNNVEEGLLDLSGRLIDQKADIDKDIKAL : 523
USPA1_O35E : INNIYELAQQQDQHSSDIKTLKN---------- : 505
USPA1_TTA2 : INNIYELAQQQDQHSSDIKTLKNNVEHGLLDLSGRLIDQKADIDKDIKTL : 542
USPA1_V117 : INNIYELAQQQDQHSSDIKTLKNNVEEGLLDLSGRLIDQKADIDKDIKTL : 513
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* 620 * 640 *
USPA1_O12E : --NVEEGLLDLSGRLIDQKADIAKNQADIAQNQIDIQDLAAYNELQDAYA : 573
USPA1_P44 : NNVEEGLLDLSGRLIDQKADIAKNQADIAQNQIDIQDLAAYNELQDAYA : 564
USPA1_ATCC : SNVEEGLLDLSGRLIDQKADIAKNQADIAQNQIDIQDLAAYNELQDAYA : 514
USPA1_O46E : NNVEEGLLDLSGRLIDQKADIAKNQADIAQNQIDIQDLAAYNELQDQYA : 543
USPA1_TTA3 : NNVEEGLLDLSGRLIDQKADIAK-------NQADIQDLAAYNELQDQYA : 566
USPA1_O35E : --NVEEGLLDLSGRLIDQKADIAKNQADIAQNQIDIQDLAAYNELQDQYA : 553
USPA1_TTA2 : KNNVEEGLLDLSGRLIDQKADIAKNQADIAQNQIDIQDLAAYNELQDQYA : 592
USPA1_V117 : SNVEEGLLDLSGRLIDQKADIAKNQADIAQNQIDIQDLAAYNELQDQYA : 563
660 * 680 * 700
USPA1_O12E : KQQTEAIDALNKASSANTDRIATAELGTAENKKDAQTAKAQANENKDGTA : 623
USPA1_P44 : KQQTEAIDALNKASSANTDRIATAELGTAENKKDAQTAKAQANENKDGTA : 614
USPA1_ATCC : KQQTEAIDALNKASSANTDRIATAELGTAENKKDAQTAKAQANENKDGTA : 564
USPA1_O46E : QKQTEAIDALNKASSANTDRIATAELGTAENKKDAQTAKAQANENKDGTA : 593
USPA1_TTA3 : QKQTEAIDALNKASSANTDRIATAELGTAENKKDAQTAKAQANENKDGTA : 616
USPA1_O35E : QKQTEAIDALNKASSENTQ---------- : 572
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* 720 * 740 *
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USPA1_P44 : KNQADIQLHDKKTNTGTLHSMVARAVGNNTQGVATNKADIAKNQADIAN : 664
USPA1_ATCC : KNQADIQLHDKKTNTGTLHSMVARAVGNNTQGVATNKADIAKNQADIAN : 614
USPA1_O46E : KNQADIQLHDKKTNTGTLHSMVARAVGNNTQGVATNKADIAKNQADIAN : 643
USPA1_TTA3 : K----------NQADIAN : 624
USPA1_O35E : ----------NIAKNQADIAN : 583
USPA1_TTA2 : KNQADIQLHDKKTNTGTLHSMVARAVGNNTQGVATNKADIAKNQADIAN : 692
USPA1_V117 : KNQADIQLHDKKTNTGTLHSMVARAVGNNTQGVATNKADIAKNQADIAN : 663
760 * 780 * 800
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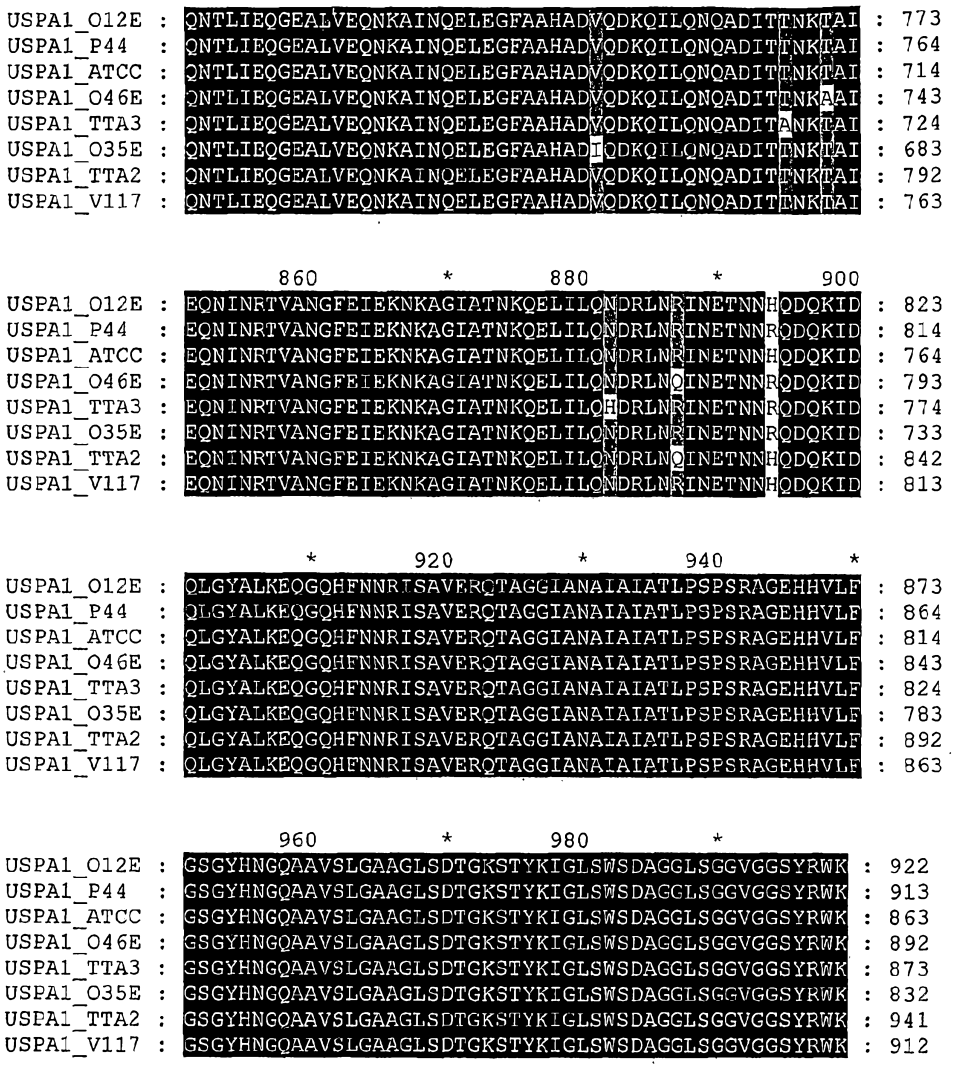
USPA1_ATCC : NIKNIYELAQQQDQHSSDIKTLAKVSAANTDRIAKNKAEADASFETLTKN : 664
USPA1_O46E : NIKNIYELAQQQDQHSSDIKTLAKVSAANTDRIAKNKAEADASFETLTKN : 693
USPA1_TTA3 : NIKNIYELAQQQDQHSSDIKTLAKVSAANTDRIAKNKAEADASFETLTKN : 674
USPA1_O35E : NINNIYELAQQQDQHSSDIKTLAKVSAANTDRIAKNKAEADASFETLTKN : 633
USPA1_TTA2 : NIKNIYELAQQQDQHSSDIKTLAKVSAANTDRIAKNKAEADASFETLTKN : 742
USPA1_V117 : NIKNIYELAQQQDQHSSDIKTLAKVSAANTDRIAKNKAEADASFETLTKN : 713
* 820 * 840 *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USPA1_P44 : QNTLIEQGEALVEQNKAINQELEGFAAHADVQDKQILQNQADITTNKTAI : 764
USPA1_ATCC : QNTLIEQGEALVEQNKAINQELEGFAAHADVQDKQILQNQADITTNKTAI : 714
USPA1_O46E : QNTLIEQGEALVEQNKAINQELEGFAAHADVQDKQILQNQADITTNKAAI : 743
USPA1_TTA3 : QNTLIEQGEALVEQNKAINQELEGFAAHADVQDKQILQNQADITANKTAI : 724
USPA1_O35E : QNTLIEQGEALVEQNKAINQELEGFAAHADIQDKQILQNQADITTNKTAI : 683
USPA1_TTA2 : QNTLIEQGEALVEQNKAINQELEGFAAHADVQDKQILQNQADITTNKTAI : 792
USPA1_V117 : QNTLIEQGEALVEQNKAINQELEGFAAHADVQDKQILQNQADITTNKTAI : 763
860 * 880 * 900
USPA1_O12E : EQNINRTVANGFEIEKNKAGIATNKQELILQNDRLNRINETNNHQDQKID : 823
USPA1_P44 : EQNINRTVANGFEIEKNKAGIATNKQELILQNDRLNRINETNNRQDQKID : 814
USPA1_ATCC : EQNINRTVANGFEIEKNKAGIATNKQELILQNDRLNRINETNNHQDQKID : 764
USPA1_O46E : EQNINRTVANGFEIEKNKAGIATNKQELILQNDRLNQINETNNRQDQKID : 793
USPA1_TTA3 : EQNINRTVANGFEIEKNKAGIATNKQELILQHDRLNRINETNNRQDQKID : 774
USPA1_O35E : EQNINRTVANGFEIEKNKAGIATNKQELILQNDRLNRINETNNRQDQKID : 733
USPA1_TTA2 : EQNINRTVANGFEIEKNKAGIATNKQELILQNDRLNQINETNNHQDQKID : 842
USPA1_V117 : EQNINRTVANGFEIEKNKAGIATNKQELILQNDRLNRINETNNHQDQKID : 813
* 920 * 940 *
USPA1_O12E : QLGYALKEQGQHFNNRISAVERQTAGGIANAIAIATLPSPSRAGEHHVLF : 873
USPA1_P44 : QLGYALKEQGQHFNNRISAVERQTAGGIANAIAIATLPSPSRAGEHHVLF : 864
USPA1_ATCC : QLGYALKEQGQHFNNRISAVERQTAGGIANAIAIATLPSPSRAGEHHVLF : 814
USPA1_O46E : QLGYALKEQGQHFNNRISAVERQTAGGIANAIAIATLPSPSRAGEHHVLF : 843
USPA1_TTA3 : QLGYALKEQGQHFNNRISAVERQTAGGIANAIAIATLPSPSRAGEHHVLF : 824
USPA1_O35E : QLGYALKEQGQHFNNRISAVERQTAGGIANAIAIATLPSPSRAGEHHVLF : 783
USPA1_TTA2 : QLGYALKEQGQHFNNRISAVERQTAGGIANAIAIATLPSPSRAGEHHVLF : 892
USPA1_V117 : QLGYALKEQGQHFNNRISAVERQTAGGIANAIAIATLPSPSRAGEHHVLF : 863
960 * 980 *
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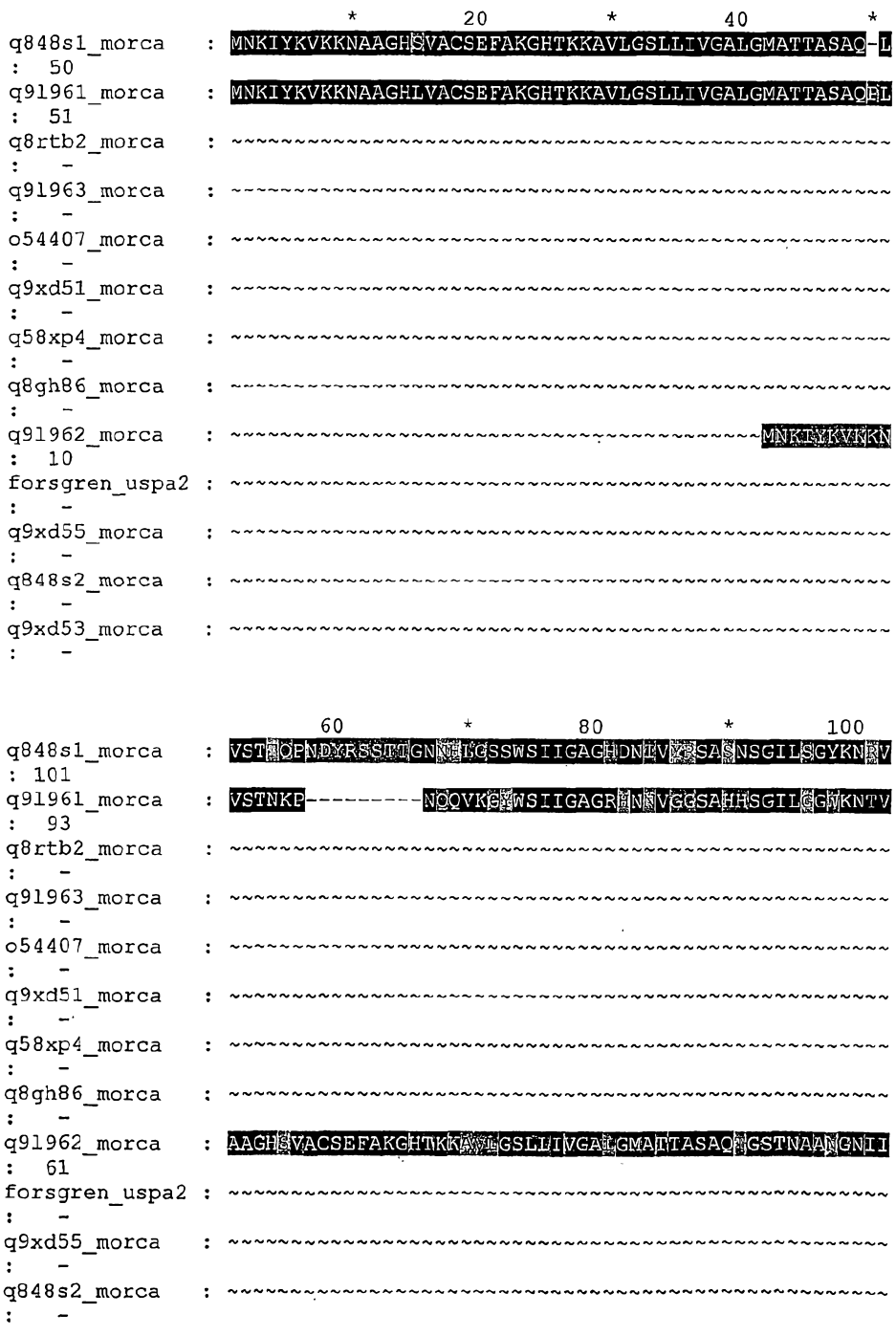
USPA1_P44 : GSGYHNGQAAVSLGAAGLSDTGKSTYKIGLSWSDAGGLSGGVGGSYRWK : 913
USPA1_ATCC : GSGYHNGQAAVSLGAAGLSDTGKSTYKIGLSWSDAGGLSGGVGGSYRWK : 863
USPA1_O46E : GSGYHNGQAAVSLGAAGLSDTGKSTYKIGLSWSDAGGLSGGVGGSYRWK : 892
USPA1_TTA3 : GSGYHNGQAAVSLGAAGLSDTGKSTYKIGLSWSDAGGLSGGVGGSYRWK : 873
USPA1_O35E : GSGYHNGQAAVSLGAAGLSDTGKSTYKIGLSWSDAGGLSGGVGGSYRWK : 832
USPA1_TTA2 : GSGYHNGQAAVSLGAAGLSDTGKSTYKIGLSWSDAGGLSGGVGGSYRWK : 941
USPA1_V117 : GSGYHNGQAAVSLGAAGLSDTGKSTYKIGLSWSDAGGLSGGVGGSYRWK : 912

Fig. 20

PileUp multiple alignment of full length UspA2 protein sequences



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                        *       120         *       140         *
q848s1_morca   : NGSTSAIVGGYDNETRGKYTFVGGGYKNLAEGHQSAIGGGYANWAEGDNAT
: 152
q91961_morca   : NGYTSAIVGGYGNETQGDYTFVGGGYKNLAKGNYTFVGGGYKNLAEGDNAT
: 144
q8rtb2_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q91963_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
o54407_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q9xd51_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q58xp4_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q8gh86_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q91962_morca   : SGVGAYVGGGVINQAKGNYPTVGGGFDNRATGNYSVISGGFDNQAKGEHST
: 112
forsgren_uspa2 : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q9xd55_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q848s2_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q9xd53_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -

                      160         *       180         *       200
q848s1_morca   : IAGGFENFAAGNQSAIGGGYANLAEGDDATIAGGFENRAEGNQSAIGGGYA
: 203
q91961_morca   : IAGGFANLAEGDNATIAGGFENRAEGIDSVVSGGYANQATGESSIVAGGSN
: 195
q8rtb2_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q91963_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
o54407_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q9xd51_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q58xp4_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q8gh86_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q91962_morca   : IAGGESNQATGRNSTVAGGSNNQAVGTNSTVAGGSNNQAKGANSEAAGVGN
: 163
forsgren_uspa2 : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q9xd55_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q848s2_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
q9xd53_morca   : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:   -
```

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                        *       220         *       240         *
q848s1_morca    : NFAAGDYTFVGGGYENRAIGNQSAIGGGYANLAEGDNATIAGGEENRAKGI
: 254
q91961_morca    : NLAEGKSSAIGGGRQNEASGDRSTVSGGYNNLAEGKSSAIGGGEENLALGN
: 246
q8rtb2_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
q91963_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
o54407_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
q9xd51_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
q58xp4_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
q8gh86_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
q91962_morca    : QANTDNAVALGKNNTINGNNSAALGSENTVNENQKNVFILGSNTTNAQSGS
: 214
forsgren_uspa2  : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
q9xd55_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
q848s2_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -
q9xd53_morca    : ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
:    -


                    260         *       280         *       300
q848s1_morca    : NSVVSGGYANQATGEESTIAGG-----FENRAEGIDSVVSGGYANQANGAS
: 300
q91961_morca    : NATISGGRQNEASGDRSIVAGG-----EQNQAIGKYSTISGGRQNEASGDR
: 292
q8rtb2_morca    : ~~MKTMKLLPLKIAVTSAMIIGLGAASTANAQ------AIETFLPNLFDNDY
:  44
q91963_morca    : ~~MKTMKLLPLKIAVTSAMMVGLGMASTANAQQQKSPKTEIFLPNLFDNDN
:  49
o54407_morca    : ~~MKTMKLLPLKIAVTSAMIVGLGAISTVNAQ------VVEQFEPNIFENEN
:  44
q9xd51_morca    : ~~MKTMKLLPLKIAVTSAMIVGLGAISTVNAQ------VVEQFEPNIFENEN
:  44
q58xp4_morca    : ~~MKTMKLLPLKIAVTSALIVGLGAASTANAQQQQKTKTEVFLPNLFYNDY
:  49
q8gh86_morca    : ~~MKTMKLLPLKIAVTSALIVGLGTASTANAQVASPANQKI--------QQK
:  42
q91962_morca    : VELGHETSGKEATAVSRARYNGLTLKNFSGVSKADNGTVSV--------SSQ
: 258
forsgren_uspa2  : ~~MKTMKLLPLKIAVTSAMIIGLGAASTANAQAKN----DI---------T
:  36
q9xd55_morca    : ~~MKTMKLLPLKIAVTSAMIIGLGAASTANAQAKN----DI---------T
:  36
q848s2_morca    : ~~MKTMKLLPLKIAVTSALIVGLGAVSTTNAQAQSRSIDQI--------QTK
:  42
q9xd53_morca    : ~~MKTMKLLPLKIAVTSAMIIGLGAASTANAQSRDRSIEDI--------QDS
:  42


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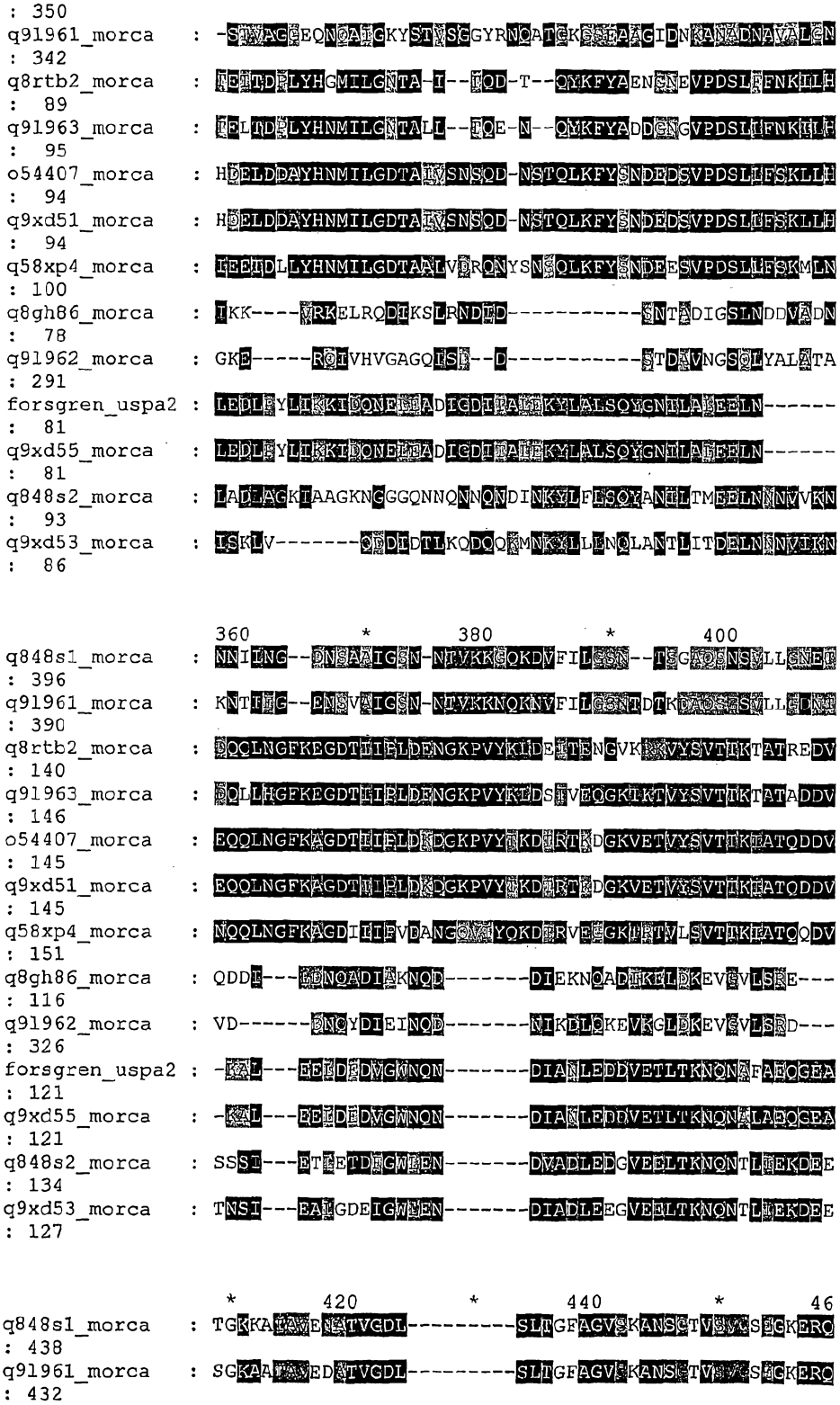
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: 89
q91963_morca : TFLTDPLYHNMILGNTALL--TQE-N--QYKFYADDGNGVPDSLTFNKTLH
: 95
o54407_morca : HDELDDAYHNMILGDTAIVSNSQD-NSTQLKFYSNDEDSVPDSLIFSKLLH
: 94
q9xd51_morca : HDELDDAYHNMILGDTAIVSNSQD-NSTQLKFYSNDEDSVPDSLIFSKLLH
: 94
q58xp4_morca : TEETDLLYHNMILGDTAALVDRQNYSNSQLKFYSNDEESVPDSLIFSKMLN
: 100
q8gh86_morca : IKK----VRKELRQDIKSLRNDLD----------SNTADIGSLNDDVADN
: 78
q91962_morca : GKE-----RQTVHVGAGQLSL--D----------STDAVNGSQLYALATA
: 291
forsgren_uspa2 : LEDLFYLIKKIDQNEIAADIGDIPALTKYLALSQYGNILAIEELN------
: 81
q9xd55_morca : LEDLFYLIKKIDQNEIAADIGDIPALEKYLALSQYGNILAIEELN------
: 81
q848s2_morca : LADLAGKTAAGKNCGGQNNQNNQNDINKYLFLSQYANILTMEELNNNVVKN
: 93
q9xd53_morca : ISKLV-------QPDEDTLKQDQQFMNKYLLINQLANTLITDELNSNVIKN
: 86

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: 396
q91961_morca : KNTTLG--ENSVAIGSN-NTVKKNQKNVFILGSNTDTKDAQSASVLLGTNT
: 390
q8rtb2_morca : DQQLNGFKEGDTIIELDENGKPVYKIDETTENGVKFKVYSVTIKTATREDV
: 140
q91963_morca : DQLLHGFKEGDTIIELDENGKPVYKLDSTVEQGKIKTVYSVTIKTATADDV
: 146
o54407_morca : EQQLNGFKAGDTIIELDKDGKPVYTKDTRTKDGKVETVYSVTIKTATQDDV
: 145
q9xd51_morca : EQQLNGFKAGDTIIELDKDGKPVYTKDTRTKDGKVETVYSVTIKTATQDDV
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q58xp4_morca : NQQLNGFKAGDIIHEVDANGQVMYQKDERVEEGKLRTVLSVTIKTATQQDV
: 151
q8gh86_morca : QDDE---LQNQADIAKNQD--------DIEKNQADIKELDKEVEVLSRE---
: 116
q91962_morca : VD------ENQYDIEINQD--------NIKDLQKEVKGLDKEVEVLSRD---
: 326
forsgren_uspa2 : -KAL---EEIDEDVGWNQN--------DIANLEDDVETLTKNQNAFAEQGEA
: 121
q9xd55_morca : -KAL---EEIDEDVGWNQN--------DIANLEDDVETLTKNQNALAEQGEA
: 121
q848s2_morca : SSSI---ETTETDEGWEEN--------DVADLEDGVEELTKNQNTLEKDEE
: 134
q9xd53_morca : TNSI---EAIGDEIGWEEN--------DIADLEEGVEELTKNQNTLEKDEE
: 127

* 420 * 440 * 46
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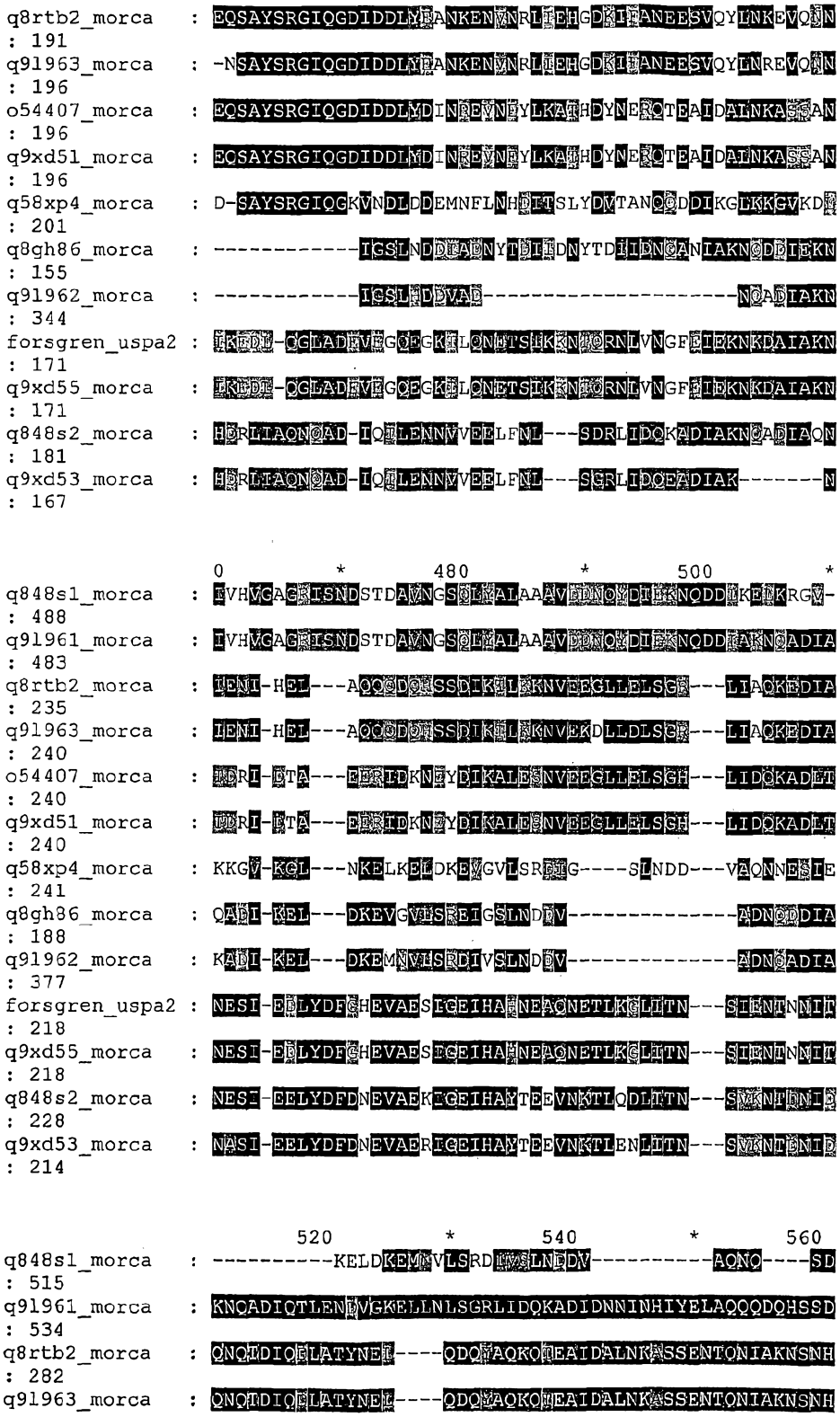
q91961_morca : SGKAAEAVEDATVGDL---------SLTGFAGVEKANSETVSVVSEGKERQ
: 432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: 196
o54407_morca : EQSAYSRGIQGDIDDLYDINREVNEYLKAEHDYNERQTEAIDALNKASSAN
: 196
q9xd51_morca : EQSAYSRGIQGDIDDLYDINREVNEYLKAEHDYNERQTEAIDALNKASSAN
: 196
q58xp4_morca : D-SAYSRGIQGKVNDLDDEMNFLNHDITSLYDVTANQQDDIKGLKKGVKDE
: 201
q8gh86_morca : ----------IGSLNDDIADNYTDIIDNYTDIIDNQANIAKNQDEIEKN
: 155
q91962_morca : ----------IGSLEDDVAD----------NQADIAKN
: 344
forsgren_uspa2 : IKEDE-QGLADEVEGQEGKELQNETSIKKNEQRNLVNGFEIEKNKDAIAKN
: 171
q9xd55_morca : IKEDE-QGLADEVEGQEGKELQNETSIKKNEQRNLVNGFEIEKNKDAIAKN
: 171
q848s2_morca : HERLIAQNQAD-IQILENNVVEELFNL---SDRLIDQKADIAKNQADIAQN
: 181
q9xd53_morca : HERLIAQNQAD-IQILENNVVEELFNL---SGRLIDQEADIAK-------N
: 167
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q848s1_morca : IVHVGAGRISNDSTDAVNGSQLEALAAAVDDNQYDIEKNQDDIKELKRGV-
: 488
q91961_morca : IVHVGAGRISNDSTDAVNGSQLEALAAAVDDNQYDIEKNQDDIAKNQADIA
: 483
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: 235
q91963_morca : IENI-HEL---AQQQDQHSSDIKELKKNVEKDLLDLSGR---LIAQKEDIA
: 240
o54407_morca : IDRI-ETA---EERIDKNEYDIKALESNVEEGLLELSGH---LIDQKADLT
: 240
q9xd51_morca : IDRI-ETA---EERIDKNEYDIKALESNVEEGLLELSGH---LIDQKADLT
: 240
q58xp4_morca : KKGV-KGL---NKELKELDKEVGVLSREIG----SLNDD---VAQNNESIE
: 241
q8gh86_morca : QADI-KEL---DKEVGVLSREIGSLNDEV----------ADNQQDIA
: 188
q91962_morca : KADI-KEL---DKEMNVLSRDIVSLNDEV----------ADNQADIA
: 377
forsgren_uspa2 : NESI-EDLYDFGHEVAESIGEIHAENEAQNETLKGLITN---SIENTNNIT
: 218
q9xd55_morca : NESI-EDLYDFGHEVAESIGEIHAENEAQNETLKGLITN---SIENTNNIT
: 218
q848s2_morca : NESI-EELYDFDNEVAEKIGEIHAYTEEVNKTLQDLITN---SVKNTENIE
: 228
q9xd53_morca : NASI-EELYDFDNEVAERIGEIHAYTEEVNKTLENLITN---SVKNTENID
: 214
520 * 540 * 560
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q91961_morca : KNQADIQTLENDVGKELLNLSGRLIDQKADIDNNINHIYELAQQQDQHSSD
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q8rtb2_morca : QNQTDIQELATYNEL----QDQVAQKQIEAIDALNKASSENTQNIAKNSNH
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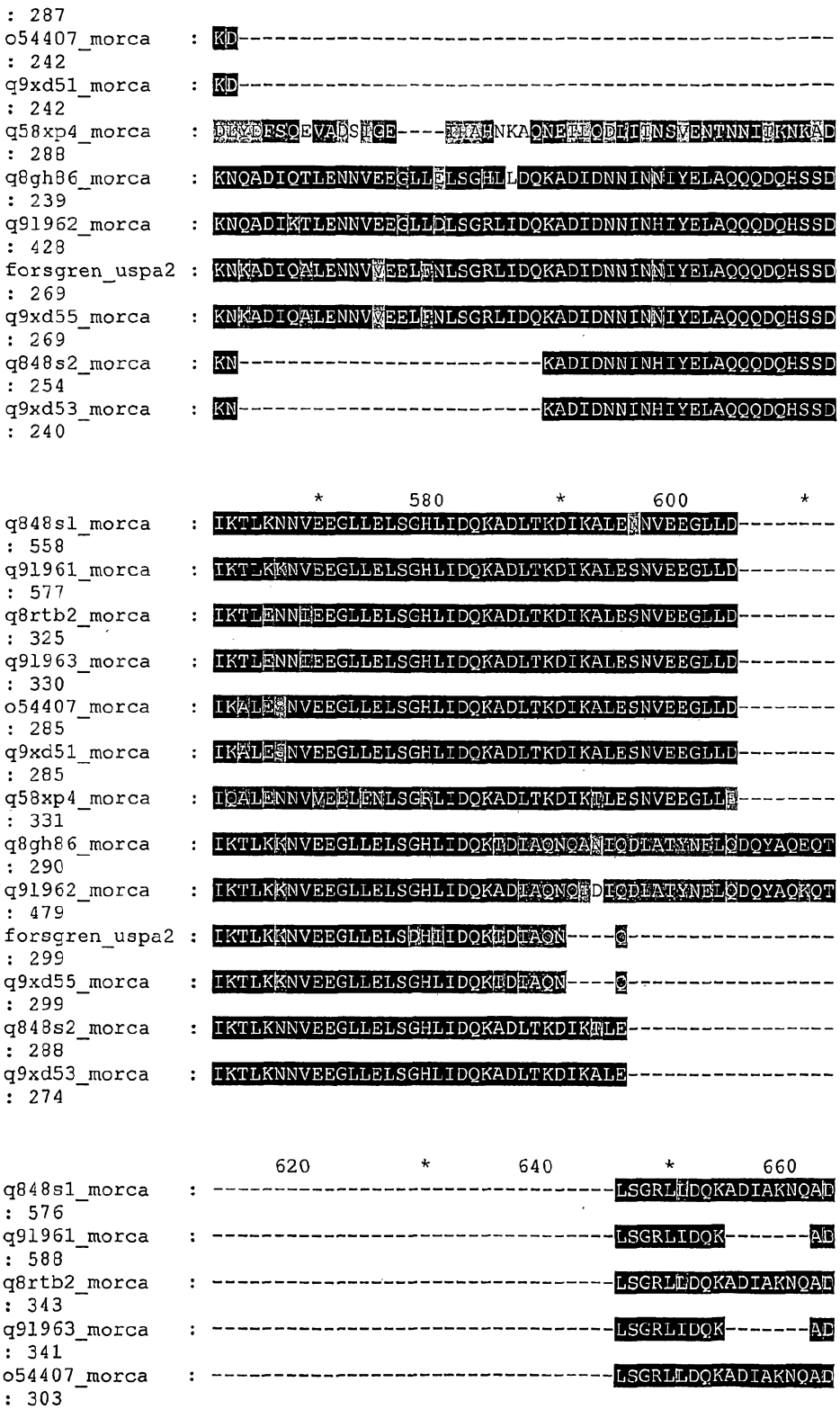
q91963_morca : QNQTDIQELATYNEL----QDQVAQKQIEAIDALNKASSENTQNIAKNSNH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: 242
q9xd51_morca : KD
: 242
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: 288
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: 239
q91962_morca : KNQADIKTLENNVEEGLLDLSGRLIDQKADIDNNINHIYELAQQQDQHSSD
: 428
forsgren_uspa2 : KNKADIQALENNVVEELENLSGRLIDQKADIDNNINNIYELAQQQDQHSSD
: 269
q9xd55_morca : KNKADIQALENNVVEELENLSGRLIDQKADIDNNINNIYELAQQQDQHSSD
: 269
q848s2_morca : KN----------KADIDNNINHIYELAQQQDQHSSD
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q9xd53_morca : KN----------KADIDNNINHIYELAQQQDQHSSD
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: 558
q91961_morca : IKTLKKNVEEGLLELSGHLIDQKADLTKDIKALESNVEEGLLD--------
: 577
q8rtb2_morca : IKTLENNIEEGLLELSGHLIDQKADLTKDIKALESNVEEGLLD--------
: 325
q91963_morca : IKTLENNIEEGLLELSGHLIDQKADLTKDIKALESNVEEGLLD--------
: 330
o54407_morca : IKALESNVEEGLLELSGHLIDQKADLTKDIKALESNVEEGLLD--------
: 285
q9xd51_morca : IKALESNVEEGLLELSGHLIDQKADLTKDIKALESNVEEGLLD--------
: 285
q58xp4_morca : IQALENNVVEELENLSGRLIDQKADLTKDIKTLESNVEEGLLE--------
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q8gh86_morca : IKTLKKNVEEGLLELSGHLIDQKTDIAQNQANIQDLATYNELQDQYAQEQT
: 290
q91962_morca : IKTLKKNVEEGLLELSGHLIDQKADIAQNQADIQDLATYNELQDQYAQKQT
: 479
forsgren_uspa2 : IKTLKKNVEEGLLELSDHIIDQKTDIAQN----Q----------------
: 299
q9xd55_morca : IKTLKKNVEEGLLELSGHLIDQKIDIAQN----Q----------------
: 299
q848s2_morca : IKTLKNNVEEGLLELSGHLIDQKADLTKDIKTLE----------------
: 288
q9xd53_morca : IKTLKNNVEEGLLELSGHLIDQKADLTKDIKALE----------------
: 274
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: 576
q91961_morca : ----------LSGRLIDQK-------AD
: 588
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o54407_morca : ----------LSGRLLDQKADIAKNQAD
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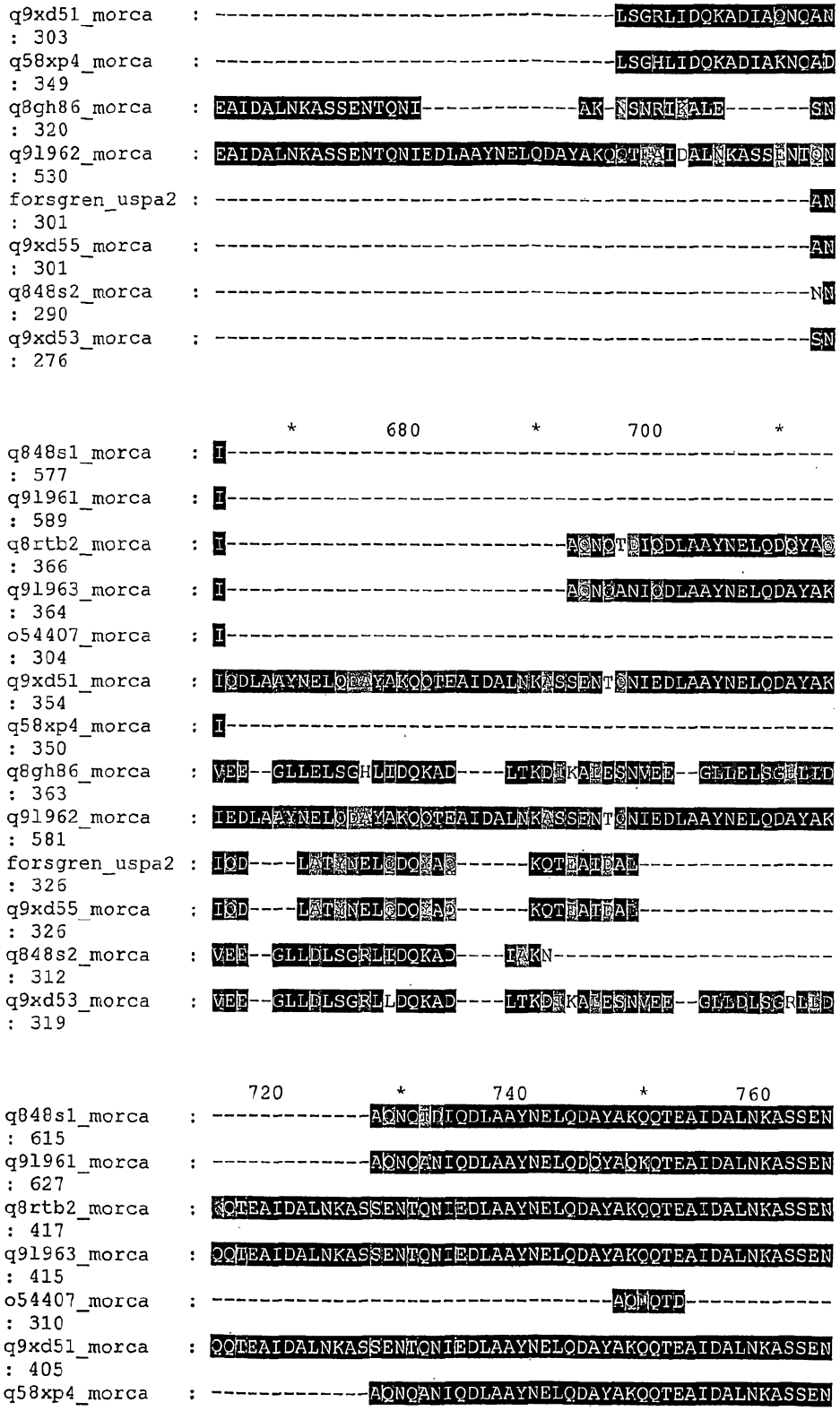
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q9xd53_morca : SN : 276
680
700
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q91961_morca : I : 589
q8rtb2_morca : I AQNQTDIQDLAAYNELQDQYAQ : 366
q91963_morca : I AQNQANIQDLAAYNELQDAYAK : 364
o54407_morca : I : 304
q9xd51_morca : IQDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIEDLAAYNELQDAYAK : 354
q58xp4_morca : I : 350
q8gh86_morca : VEE GLLELSGHLIDQKAD LTKDIKALESNVEE GLLELSGLLID : 363
q91962_morca : IEDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIEDLAAYNELQDAYAK : 581
forsgren_uspa2 : IQD LATQNELQDQYAQ KQTEAIDAL : 326
q9xd55_morca : IQD LATQNELQDQYAQ KQTEAIDAL : 326
q848s2_morca : VEE GLLDLSGRLIDQKAD IAKN : 312
q9xd53_morca : VEE GLLDLSGRLLDQKAD LTKDIKALESNVEE GLLDLSGRLLD : 319
720
740
760
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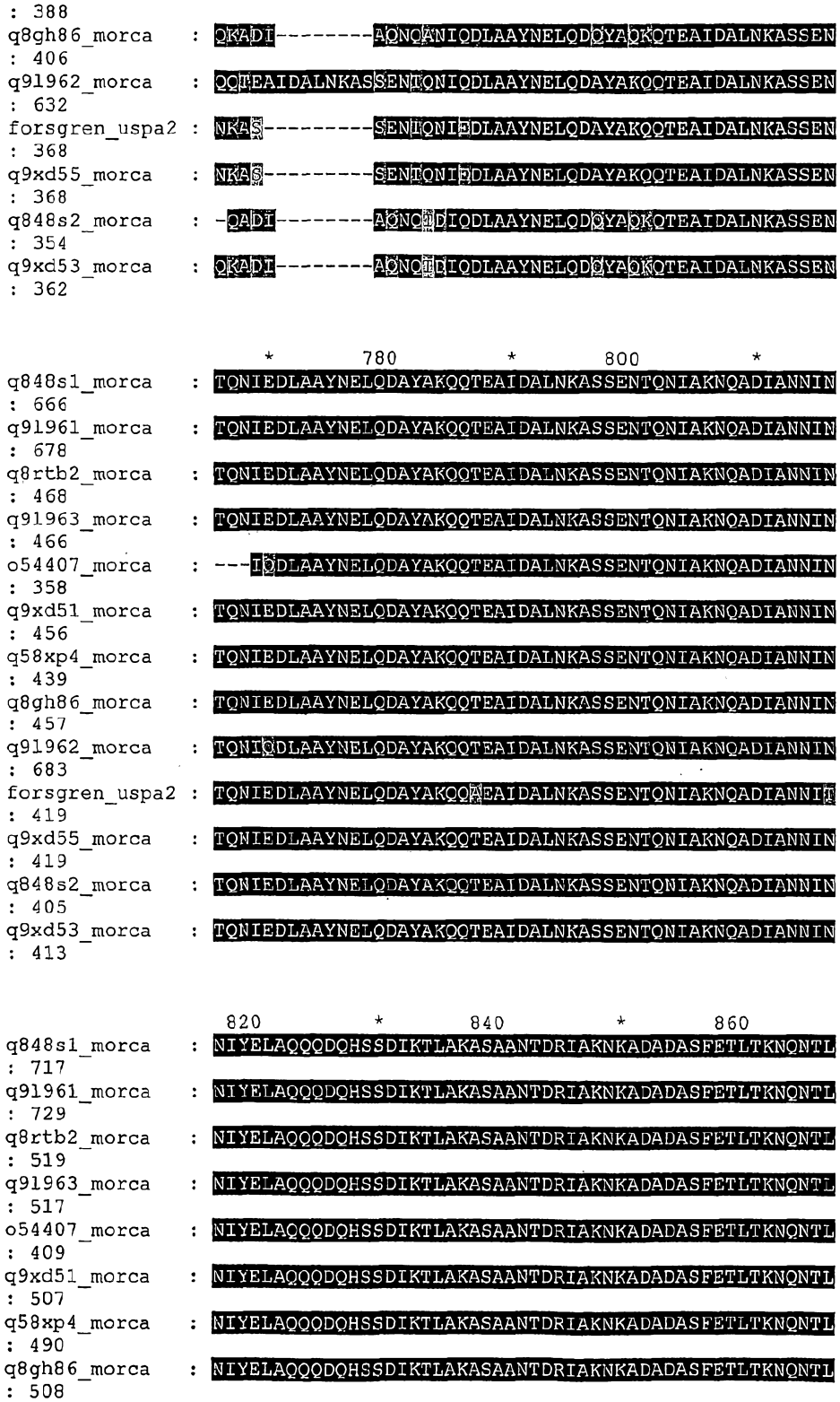
q91961_morca : AQNQANIQDLAAYNELQDQYAQKQTEAIDALNKASSEN : 627
q8rtb2_morca : QQTEAIDALNKASSENTQNIEDLAAYNELQDAYAKQQTEAIDALNKASSEN : 417
q91963_morca : QQTEAIDALNKASSENTQNIEDLAAYNELQDAYAKQQTEAIDALNKASSEN : 415
o54407_morca : AQNQTD : 310
q9xd51_morca : QQTEAIDALNKASSENTQNIEDLAAYNELQDAYAKQQTEAIDALNKASSEN : 405
q58xp4_morca : AQNQANIQDLAAYNELQDAYAKQQTEAIDALNKASSEN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: 632
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: 368
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: 368
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: 354
q9xd53_morca : QKADI--------AQNQTDIQDLAAYNELQDQYAQKQTEAIDALNKASSEN
: 362
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q91961_morca : TQNIEDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIAKNQADIANNIN
: 678
q8rtb2_morca : TQNIEDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIAKNQADIANNIN
: 468
q91963_morca : TQNIEDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIAKNQADIANNIN
: 466
o54407_morca : ---IQDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIAKNQADIANNIN
: 358
q9xd51_morca : TQNIEDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIAKNQADIANNIN
: 456
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: 439
q8gh86_morca : TQNIEDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIAKNQADIANNIN
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q91962_morca : TQNIQDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIAKNQADIANNIN
: 683
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q9xd53_morca : TQNIEDLAAYNELQDAYAKQQTEAIDALNKASSENTQNIAKNQADIANNIN
: 413
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: 717
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: 729
q8rtb2_morca : NIYELAQQQDQHSSDIKTLAKASAANTDRIAKNKADADASFETLTKNQNTL
: 519
q91963_morca : NIYELAQQQDQHSSDIKTLAKASAANTDRIAKNKADADASFETLTKNQNTL
: 517
o54407_morca : NIYELAQQQDQHSSDIKTLAKASAANTDRIAKNKADADASFETLTKNQNTL
: 409
q9xd51_morca : NIYELAQQQDQHSSDIKTLAKASAANTDRIAKNKADADASFETLTKNQNTL
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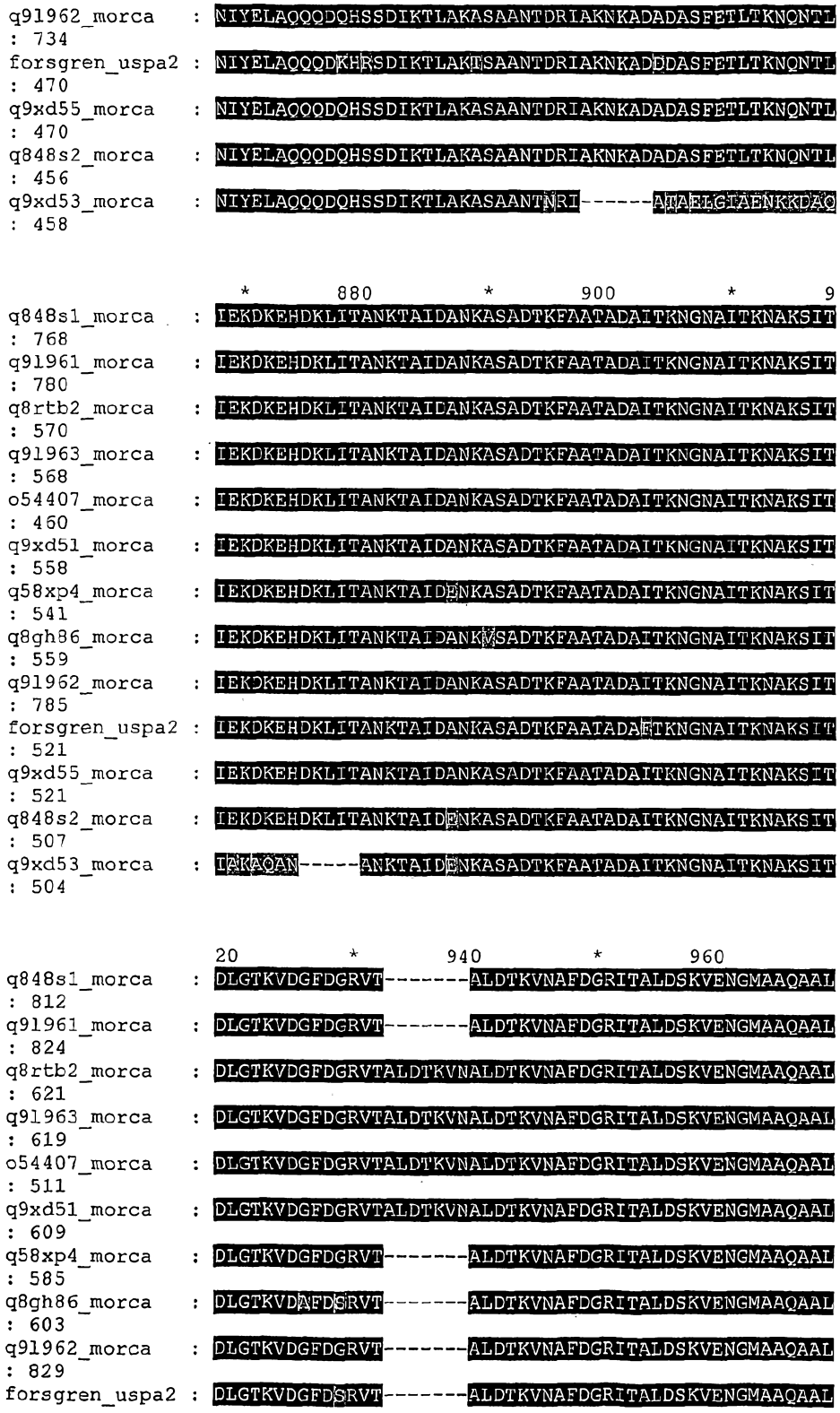
: 508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* 880 * 900 * 9
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q91961_morca : IEKDKEHDKLITANKTAIDANKASADTKFAATADAITKNGNAITKNAKSIT : 780
q8rtb2_morca : IEKDKEHDKLITANKTAIDANKASADTKFAATADAITKNGNAITKNAKSIT : 570
q91963_morca : IEKDKEHDKLITANKTAIDANKASADTKFAATADAITKNGNAITKNAKSIT : 568
o54407_morca : IEKDKEHDKLITANKTAIDANKASADTKFAATADAITKNGNAITKNAKSIT : 460
q9xd51_morca : IEKDKEHDKLITANKTAIDANKASADTKFAATADAITKNGNAITKNAKSIT : 558
q58xp4_morca : IEKDKEHDKLITANKTAIDENKASADTKFAATADAITKNGNAITKNAKSIT : 541
q8gh86_morca : IEKDKEHDKLITANKTAIDANKVSADTKFAATADAITKNGNAITKNAKSII : 559
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q9xd55_morca : IEKDKEHDKLITANKTAIDANKASADTKFAATADAITKNGNAITKNAKSIT : 521
q848s2_morca : IEKDKEHDKLITANKTAIDENKASADTKFAATADAITKNGNAITKNAKSIT : 507
q9xd53_morca : IAKAQAN-----ANKTAIDENKASADTKFAATADAITKNGNAITKNAKSIT : 504
20 * 940 * 960
q848s1_morca : DLGTKVDGFDGRVT-------ALDTKVNAFDGRITALDSKVENGMAAQAAL : 812
q91961_morca : DLGTKVDGFDGRVT-------ALDTKVNAFDGRITALDSKVENGMAAQAAL : 824
q8rtb2_morca : DLGTKVDGFDGRVTALDTKVNALDTKVNAFDGRITALDSKVENGMAAQAAL : 621
q91963_morca : DLGTKVDGFDGRVTALDTKVNALDTKVNAFDGRITALDSKVENGMAAQAAL : 619
o54407_morca : DLGTKVDGFDGRVTALDTKVNALDTKVNAFDGRITALDSKVENGMAAQAAL : 511
q9xd51_morca : DLGTKVDGFDGRVTALDTKVNALDTKVNAFDGRITALDSKVENGMAAQAAL : 609
q58xp4_morca : DLGTKVDGFDGRVT-------ALDTKVNAFDGRITALDSKVENGMAAQAAL : 585
q8gh86_morca : DLGTKVDAFDSRVT-------ALDTKVNAFDGRITALDSKVENGMAAQAAL : 603
q91962_morca : DLGTKVDGFDGRVT-------ALDTKVNAFDGRITALDSKVENGMAAQAAL : 829
forsgren_uspa2 : DLGTKVDGFDSRVT-------ALDTKVNAFDGRITALDSKVENGMAAQAAL

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: 565
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q848s2_morca    : DLGTKVDGFDGRVT--------ALDTKVNAFDGRITALDSKVENGMAAQAAL
: 551
q9xd53_morca    : DLGTKVDGFDGRVT--------ALDTKVNAFDGRITALDSKVENGMAAQAAL
: 548

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: 863
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: 875
q8rtb2_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 672
q91963_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 670
o54407_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 562
q9xd51_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 660
q58xp4_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 636
q8gh86_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 654
q91962_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 880
forsgren_uspa2  : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 616
q9xd55_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
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q848s2_morca    : SGLFQPYSVGKFNATAALGGYGSKSAVAIGAGYRVNPNLAFKAGAAINTSG
: 602
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: 599

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q8rtb2_morca    : NKKGSYNIGVNYEF : 686
q91963_morca    : NKKGSYNIGVNYEF : 684
o54407_morca    : NKKGSYNIGVNYEF : 576
q9xd51_morca    : NKKGSYNIGVNYEF : 674
q58xp4_morca    : NKKGSYNIGVNYEF : 650
q8gh86_morca    : NKKGSYNIGVNYEF : 668
q91962_morca    : NKKGSYNIGVNYEF : 894
forsgren_uspa2  : NKKGSYNIGVNYEF : 630
q9xd55_morca    : NKKGSYNIGVNYEF : 630
q848s2_morca    : NKKGSYNIGVNYEF : 616
q9xd53_morca    : NKKGSYNIGVNYEF : 613
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Fig. 21

% identity in regions identified on Forsgren sequence

	30-177 laminin binding	165-318 fibronectin binding	302-458 C3-binding
q848s1_morca	8	19	74
q9l961_morca	6	41	73
q8rtb2_morca	9	22	67
q9l963_morca	10	23	68
o54407_morca	10	17	53
q9xd51_morca	10	19	66
q58xp4_morca	10	16	75
q8gh86_morca	18	43	69
q9l962_morca	9	36	64
q9xd55_morca	99	98	96
q848s2_morca	35	54	74
q9xd53_morca	29	52	64

SEQUENCE LISTING

<110> Arne Forsgren AB

<120> INTERACTION OF MORAXELLA CATARRHALIS TO EPITHELIAL CELLS...

<130> 21027432

<160> 10

<170> PatentIn version 3.1

<210> 1

<211> 31

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA1 405-435 or UspA2 244-274) or synthetic petide

<400> 1

Asp Gln Lys Ala Asp Ile Asp Asn Asn Ile Asn Asn Ile Tyr Glu Leu
1 5 10 15

Ala Gln Gln Gln Asp Gln His Ser Ser Asp Ile Lys Thr Leu Lys
20 25 30

<210> 2

<211> 154

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA1 299-452) or synthetic peptide

<400> 2

Thr Gly Asn Gly Thr Val Ser Val Gly Lys Lys Gly Lys Glu Arg Gln
1 5 10 15

Ile Val His Val Gly Ala Gly Glu Ile Ser Asp Thr Ser Thr Asp Ala Val Asn
20 25 30

Gly Ser Gln Leu His Val Leu Ala Thr Val Val Ala Gln Asn Lys Ala
35 40 45 50

Asp Ile Lys Asp Leu Asp Asp Glu Val Gly Leu Leu Gly Glu Glu Ile
55 60 65

Asn Ser Leu Glu Gly Glu Ile Phe Asn Asn Gln Asp Ala Ile Ala Lys
70 75 80

Asn Gln Ala Asp Ile Lys Thr Leu Glu Ser Asn Val Glu Glu Gly Leu
85 90 95

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100 105 110

Asp Asn Asn Ile Asn Asn Ile Tyr Glu Leu Ala Gln Gln Gln Asp Gln
115 120 125

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

Leu Asp Leu Ser Gly Arg Leu Ile Asp Gln
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<210> 3

<211> 154

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<400> 3

Lys Asp Ala Ile Ala Lys Asn Asn Glu Ser Ile Glu Asp Leu Tyr Asp
1 5 10 15

Phe Gly His Glu Val Ala Glu Ser Ile Gly Glu Ile His Ala His Asn
20 25 30

Glu Ala Gln Asn Glu Thr Leu Lys Gly Leu Ile Thr Asn Ser Ile Glu
35 40 45

Asn Thr Asn Asn Ile Thr Lys Asn Lys Ala Asp Ile Gln Ala Leu Glu
50 55 60

Asn Asn Val Val Glu Glu Leu Phe Asn Leu Ser Gly Arg Leu Ile Asp
65 70 75 80

Gln Lys Ala Asp Ile Asp Asn Asn Ile Asn Asn Ile Tyr Glu Leu Ala
85 90 95

Gln Gln Gln Asp Gln His Ser Ser Asp Ile Lys Thr Leu Lys Lys Asn
100 105 110

Val Glu Glu Gly Leu Leu Glu Leu Ser Asp His Ile Ile Asp Gln Lys
115 120 125

Thr Asp Ile Ala Gln Asn Gln Ala Asn Ile Gln Asp Leu Ala Thr Tyr
130 135 140

Asn Glu Leu Gln Asp Gln Tyr Ala Gln Lys
145 150

<210> 4

<211> 721

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA1 50-770) or synthetic peptide

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1 5 10 15

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

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

Asn Glu Tyr Ser Thr Ile Gly Ser Gly Gly Tyr Asn Lys Ala Lys Gly
50 55 60

Arg Tyr Ser Thr Ile Gly Gly Gly Gly Tyr Asn Glu Ala Thr Asn Gln
65 70 75 80

Tyr Ser Thr Ile Gly Gly Gly Asp Asn Asn Thr Ala Lys Gly Arg Tyr
85 90 95

Ser Thr Ile Gly Gly Gly Gly Tyr Asn Glu Ala Thr Ile Glu Asn Ser
100 105 110

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115 120 125

Val Ala Gly Gly Tyr Asn Asn Glu Ala Thr Gly Thr Asp Ser Thr Ile
130 135 140

Ala Gly Gly Arg Lys Asn Gln Ala Thr Gly Lys Gly Ser Phe Ala Ala
145 150 155 160

Gly Ile Asp Asn Lys Ala Asn Ala Asp Asn Ala Val Ala Leu Gly Asn
165 170 175

Lys Asn Thr Ile Glu Gly Glu Asn Ser Val Ala Ile Gly Ser Asn Asn
180 185 190

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

Asp Thr Thr Asn Ala Gln Asn Gly Ser Val Leu Leu Gly His Asn Thr
210 215 220

Ala Gly Lys Ala Ala Thr Ile Val Asn Ser Ala Glu Val Gly Gly Leu
225 230 235 240

Ser Leu Thr Gly Phe Ala Gly Ala Ser Lys Thr Gly Asn Gly Thr Val
245 250 255

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

Gly Glu Ile Ser Asp Thr Ser Thr Asp Ala Val Asn Gly Ser Gln Leu
275 280 285

His Val Leu Ala Thr Val Val Ala Gln Asn Lys Ala Asp Ile Lys Asp
290 295 300

Leu Asp Asp Glu Val Gly Leu Leu Gly Glu Glu Ile Asn Ser Leu Glu
305 310 315 320

Gly Glu Ile Phe Asn Asn Gln Asp Ala Ile Ala Lys Asn Gln Ala Asp
325 330 335

Ile Lys Thr Leu Glu Ser Asn Val Glu Glu Gly Leu Leu Asp Leu Ser
340 345 350

Gly Arg Leu Leu Asp Gln Lys Ala Asp Ile Asp Asn Asn Ile Asn Asn
355 360 365

Ile Tyr Glu Leu Ala Gln Gln Gln Asp Gln His Ser Ser Asp Ile Lys
370 375 380

Thr Leu Lys Asn Asn Val Glu Glu Gly Leu Leu Asp Leu Ser Gly Arg
385 390 395 400

Leu Ile Asp Gln Lys Ala Asp Leu Thr Lys Asp Ile Lys Ala Leu Glu
405 410 415

Ser Asn Val Glu Glu Gly Leu Leu Asp Leu Ser Gly Arg Leu Ile Asp
420 425 430

Gln Lys Ala Asp Ile Ala Lys Asn Gln Ala Asp Ile Ala Gln Asn Gln
435 440 445

Thr Asp Ile Gln Asp Leu Ala Ala Tyr Asn Glu Leu Gln Asp Ala Tyr
450 455 460

Ala Lys Gln Gln Thr Glu Ala Ile Asp Ala Leu Asn Lys Ala Ser Ser
465 470 475 480

Ala Asn Thr Asp Arg Ile Ala Thr Ala Glu Leu Gly Ile Ala Glu Asn
485 490 495

Lys Lys Asp Ala Gln Ile Ala Lys Ala Gln Ala Asn Glu Asn Lys Asp
500 505 510

Gly Ile Ala Lys Asn Gln Ala Asp Ile Gln Leu His Asp Lys Lys Ile
515 520 525

Thr Asn Leu Gly Ile Leu His Ser Met Val Ala Arg Ala Val Gly Asn
530 535 540

Asn Thr Gln Gly Val Ala Thr Asn Lys Ala Asp Ile Ala Lys Asn Gln
545 550 555 560

Ala Asp Ile Ala Asn Asn Ile Lys Asn Ile Tyr Glu Leu Ala Gln Gln
565 570 575

Gln Asp Gln His Ser Ser Asp Ile Lys Thr Leu Ala Lys Val Ser Ala
580 585 590

Ala Asn Thr Asp Arg Ile Ala Lys Asn Lys Ala Glu Ala Asp Ala Ser
595 600 605

Phe Glu Thr Leu Thr Lys Asn Gln Asn Thr Leu Ile Glu Gln Gly Glu
610 615 620

Ala Leu Val Glu Gln Asn Lys Ala Ile Asn Gln Glu Leu Glu Gly Phe
625 630 635 640

Ala Ala His Ala Asp Val Gln Asp Lys Gln Ile Leu Gln Asn Gln Ala
645 650 655

Asp Ile Thr Thr Asn Lys Thr Ala Ile Glu Gln Asn Ile Asn Arg Thr
660 665 670

Val Ala Asn Gly Phe Glu Ile Glu Lys Asn Lys Ala Gly Ile Ala Thr
675 680 685

Asn Lys Gln Glu Leu Ile Leu Gln Asn Asp Arg Leu Asn Arg Ile Asn
690 695 700

```
Glu Thr Asn Asn His Gln Asp Gln Lys Ile Asp Gln Leu Gly Tyr Ala
705                 710                 715                 720

Leu
```

<210> 5

<211> 443

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA1 50-491) or synthetic peptide

<400> 5

```
Lys Val Gly Lys Ala Thr Asn Lys Ile Ser Gly Gly Asp Asn Asn Thr
1               5                   10                  15

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

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

Asn Glu Tyr Ser Thr Ile Gly Ser Gly Gly Tyr Asn Lys Ala Lys Gly
    50                  55                  60

Arg Tyr Ser Thr Ile Gly Gly Gly Gly Tyr Asn Glu Ala Thr Asn Gln
65                  70                  75                  80

Tyr Ser Thr Ile Gly Gly Gly Asp Asn Asn Thr Ala Lys Gly Arg Tyr
                85                  90                  95

Ser Thr Ile Gly Gly Gly Gly Tyr Asn Glu Ala Thr Ile Glu Asn Ser
            100                 105                 110

Thr Val Gly Gly Gly Gly Tyr Asn Gln Ala Lys Gly Arg Asn Ser Thr
        115                 120                 125

Val Ala Gly Gly Tyr Asn Asn Glu Ala Thr Gly Thr Asp Ser Thr Ile
    130                 135                 140

Ala Gly Gly Arg Lys Asn Gln Ala Thr Gly Lys Gly Ser Phe Ala Ala
145                 150                 155                 160

Gly Ile Asp Asn Lys Ala Asn Ala Asp Asn Ala Val Ala Leu Gly Asn
                165                 170                 175

Lys Asn Thr Ile Glu Gly Glu Asn Ser Val Ala Ile Gly Ser Asn Asn
```

180 185 190

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

Asp Thr Thr Asn Ala Gln Asn Gly Ser Val Leu Leu Gly His Asn Thr
210 215 220

Ala Gly Lys Ala Ala Thr Ile Val Asn Ser Ala Glu Val Gly Gly Leu
225 230 235 240

Ser Leu Thr Gly Phe Ala Gly Ala Ser Lys Thr Gly Asn Gly Thr Val
245 250 255

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

Gly Glu Ile Ser Asp Thr Ser Thr Asp Ala Val Asn Gly Ser Gln Leu
275 280 285

His Val Leu Ala Thr Val Val Ala Gln Asn Lys Ala Asp Ile Lys Asp
290 295 300

Leu Asp Asp Glu Val Gly Leu Leu Gly Glu Glu Ile Asn Ser Leu Glu
305 310 315 320

Gly Glu Ile Phe Asn Asn Gln Asp Ala Ile Ala Lys Asn Gln Ala Asp
325 330 335

Ile Lys Thr Leu Glu Ser Asn Val Glu Glu Gly Leu Leu Asp Leu Ser
340 345 350

Gly Arg Leu Leu Asp Gln Lys Ala Asp Ile Asp Asn Asn Ile Asn Asn
355 360 365

Ile Tyr Glu Leu Ala Gln Gln Gln Asp Gln His Ser Ser Asp Ile Lys
370 375 380

Thr Leu Lys Asn Asn Val Glu Glu Gly Leu Leu Asp Leu Ser Gly Arg
385 390 395 400

Leu Ile Asp Gln Lys Ala Asp Leu Thr Lys Asp Ile Lys Ala Leu Glu
405 410 415

Ser Asn Val Glu Glu Gly Leu Leu Asp Leu Ser Gly Arg Leu Ile Asp
420 425 430

Gln Lys Ala Asp Ile Ala Lys Asn Gln Ala Asp
435 440

<210> 6

<211> 510

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA2 30-539) or synthetic peptide

<400> 6

```
Gln Ala Lys Asn Asp Ile Thr Leu Glu Asp Leu Pro Tyr Leu Ile Lys
1               5                  10                  15

Lys Ile Asp Gln Asn Glu Leu Glu Ala Asp Ile Gly Asp Ile Thr Ala
            20                  25                  30

Leu Glu Lys Tyr Leu Ala Leu Ser Gln Tyr Gly Asn Ile Leu Ala Leu
        35                  40                  45

Glu Glu Leu Asn Lys Ala Leu Glu Glu Leu Asp Glu Asp Val Gly Trp
    50                  55                  60

Asn Gln Asn Asp Ile Ala Asn Leu Glu Asp Asp Val Glu Thr Leu Thr
65                  70                  75                  80

Lys Asn Gln Asn Ala Phe Ala Glu Gln Gly Glu Ala Ile Lys Glu Asp
                85                  90                  95

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

Gln Asn Glu Thr Ser Ile Lys Lys Asn Thr Gln Arg Asn Leu Val Asn
        115                 120                 125

Gly Phe Glu Ile Glu Lys Asn Lys Asp Ala Ile Ala Lys Asn Asn Glu
    130                 135                 140

Ser Ile Glu Asp Leu Tyr Asp Phe Gly His Glu Val Ala Glu Ser Ile
145                 150                 155                 160

Gly Glu Ile His Ala His Asn Glu Ala Gln Asn Glu Thr Leu Lys Gly
                165                 170                 175

Leu Ile Thr Asn Ser Ile Glu Asn Thr Asn Asn Ile Thr Lys Asn Lys
            180                 185                 190

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

Leu Ser Gly Arg Leu Ile Asp Gln Lys Ala Asp Ile Asp Asn Asn Ile
    210                 215                 220
```

```
Asn Asn Ile Tyr Glu Leu Ala Gln Gln Gln Asp Gln His Ser Ser Asp
225                 230                 235                 240

Ile Lys Thr Leu Lys Lys Asn Val Glu Glu Gly Leu Leu Glu Leu Ser
                245                 250                 255

Asp His Ile Ile Asp Gln Lys Thr Asp Ile Ala Gln Asn Gln Ala Asn
            260                 265                 270

Ile Gln Asp Leu Ala Thr Tyr Asn Glu Leu Gln Asp Gln Tyr Ala Gln
        275                 280                 285

Lys Gln Thr Glu Ala Ile Asp Ala Leu Asn Lys Ala Ser Ser Glu Asn
    290                 295                 300

Thr Gln Asn Ile Glu Asp Leu Ala Ala Tyr Asn Glu Leu Gln Asp Ala
305                 310                 315                 320

Tyr Ala Lys Gln Gln Thr Glu Ala Ile Asp Ala Leu Asn Lys Ala Ser
                325                 330                 335

Ser Glu Asn Thr Gln Asn Ile Glu Asp Leu Ala Ala Tyr Asn Glu Leu
            340                 345                 350

Gln Asp Ala Tyr Ala Lys Gln Gln Ala Glu Ala Ile Asp Ala Leu Asn
        355                 360                 365

Lys Ala Ser Ser Glu Asn Thr Gln Asn Ile Ala Lys Asn Gln Ala Asp
    370                 375                 380

Ile Ala Asn Asn Ile Thr Asn Ile Tyr Glu Leu Ala Gln Gln Gln Asp
385                 390                 395                 400

Lys His Arg Ser Asp Ile Lys Thr Leu Ala Lys Thr Ser Ala Ala Asn
                405                 410                 415

Thr Asp Arg Ile Ala Lys Asn Lys Ala Asp Asp Asp Ala Ser Phe Glu
            420                 425                 430

Thr Leu Thr Lys Asn Gln Asn Thr Leu Ile Glu Lys Asp Lys Glu His
        435                 440                 445

Asp Lys Leu Ile Thr Ala Asn Lys Thr Ala Ile Asp Ala Asn Lys Ala
    450                 455                 460

Ser Ala Asp Thr Lys Phe Ala Ala Thr Ala Asp Ala Phe Thr Lys Asn
465                 470                 475                 480

Gly Asn Ala Ile Thr Lys Asn Ala Lys Ser Ile Thr Asp Leu Gly Thr
                485                 490                 495
```

Lys Val Asp Gly Phe Asp Ser Arg Val Thr Ala Leu Asp Thr
500 505 510

<210> 7

<211> 322

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA2 30-351) or synthetic peptide

<400> 7

Gln Ala Lys Asn Asp Ile Thr Leu Glu Asp Leu Pro Tyr Leu Ile Lys
1 5 10 15

Lys Ile Asp Gln Asn Glu Leu Glu Ala Asp Ile Gly Asp Ile Thr Ala
20 25 30

Leu Glu Lys Tyr Leu Ala Leu Ser Gln Tyr Gly Asn Ile Leu Ala Leu
35 40 45

Glu Glu Leu Asn Lys Ala Leu Glu Glu Leu Asp Glu Asp Val Gly Trp
50 55 60

Asn Gln Asn Asp Ile Ala Asn Leu Glu Asp Asp Val Glu Thr Leu Thr
65 70 75 80

Lys Asn Gln Asn Ala Phe Ala Glu Gln Gly Glu Ala Ile Lys Glu Asp
85 90 95

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

Gln Asn Glu Thr Ser Ile Lys Lys Asn Thr Gln Arg Asn Leu Val Asn
115 120 125

Gly Phe Glu Ile Glu Lys Asn Lys Asp Ala Ile Ala Lys Asn Asn Glu
130 135 140

Ser Ile Glu Asp Leu Tyr Asp Phe Gly His Glu Val Ala Glu Ser Ile
145 150 155 160

Gly Glu Ile His Ala His Asn Glu Ala Gln Asn Glu Thr Leu Lys Gly
165 170 175

Leu Ile Thr Asn Ser Ile Glu Asn Thr Asn Asn Ile Thr Lys Asn Lys
180 185 190

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

Leu Ser Gly Arg Leu Ile Asp Gln Lys Ala Asp Ile Asp Asn Asn Ile
210 215 220

Asn Asn Ile Tyr Glu Leu Ala Gln Gln Gln Asp Gln His Ser Ser Asp
225 230 235 240

Ile Lys Thr Leu Lys Lys Asn Val Glu Glu Gly Leu Leu Glu Leu Ser
245 250 255

Asp His Ile Ile Asp Gln Lys Thr Asp Ile Ala Gln Asn Gln Ala Asn
260 265 270

Ile Gln Asp Leu Ala Thr Tyr Asn Glu Leu Gln Asp Gln Tyr Ala Gln
275 280 285

Lys Gln Thr Glu Ala Ile Asp Ala Leu Asn Lys Ala Ser Ser Glu Asn
290 295 300

Thr Gln Asn Ile Glu Asp Leu Ala Ala Tyr Asn Glu Leu Gln Asp Ala
305 310 315 320

Tyr Ala

<210> 8

<211> 148

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA2 30-177) or synthetic peptide

<400> 8

Gln Ala Lys Asn Asp Ile Thr Leu Glu Asp Leu Pro Tyr Leu Ile Lys
1 5 10 15

Lys Ile Asp Gln Asn Glu Leu Glu Ala Asp Ile Gly Asp Ile Thr Ala
20 25 30

Leu Glu Lys Tyr Leu Ala Leu Ser Gln Tyr Gly Asn Ile Leu Ala Leu
35 40 45

Glu Glu Leu Asn Lys Ala Leu Glu Glu Leu Asp Glu Asp Val Gly Trp
50 55 60

Asn Gln Asn Asp Ile Ala Asn Leu Glu Asp Asp Val Glu Thr Leu Thr
65 70 75 80

Lys Asn Gln Asn Ala Phe Ala Glu Gln Gly Glu Ala Ile Lys Glu Asp
85 90 95

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

Gln Asn Glu Thr Ser Ile Lys Lys Asn Thr Gln Arg Asn Leu Val Asn
115 120 125

Gly Phe Glu Ile Glu Lys Asn Lys Asp Ala Ile Ala Lys Asn Asn Glu
130 135 140

Ser Ile Glu Asp
145

<210> 9

<211> 340

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA2 200-539) or synthetic peptide

<400> 9

Asn Glu Thr Leu Lys Gly Leu Ile Thr Asn Ser Ile Glu Asn Thr Asn
1 5 10 15

Asn Ile Thr Lys Asn Lys Ala Asp Ile Gln Ala Leu Glu Asn Asn Val
20 25 30

Val Glu Glu Leu Phe Asn Leu Ser Gly Arg Leu Ile Asp Gln Lys Ala
35 40 45

Asp Ile Asp Asn Asn Ile Asn Asn Ile Tyr Glu Leu Ala Gln Gln Gln
50 55 60

Asp Gln His Ser Ser Asp Ile Lys Thr Leu Lys Lys Asn Val Glu Glu
65 70 75 80

Gly Leu Leu Glu Leu Ser Asp His Ile Ile Asp Gln Lys Thr Asp Ile
85 90 95

Ala Gln Asn Gln Ala Asn Ile Gln Asp Leu Ala Thr Tyr Asn Glu Leu
100 105 110

Gln Asp Gln Tyr Ala Gln Lys Gln Thr Glu Ala Ile Asp Ala Leu Asn
115 120 125

Lys Ala Ser Ser Glu Asn Thr Gln Asn Ile Glu Asp Leu Ala Ala Tyr
130 135 140

Asn Glu Leu Gln Asp Ala Tyr Ala Lys Gln Gln Thr Glu Ala Ile Asp

145 150 155 160

Ala Leu Asn Lys Ala Ser Ser Glu Asn Thr Gln Asn Ile Glu Asp Leu
165 170 175

Ala Ala Tyr Asn Glu Leu Gln Asp Ala Tyr Ala Lys Gln Gln Ala Glu
180 185 190

Ala Ile Asp Ala Leu Asn Lys Ala Ser Ser Glu Asn Thr Gln Asn Ile
195 200 205

Ala Lys Asn Gln Ala Asp Ile Ala Asn Asn Ile Thr Asn Ile Tyr Glu
210 215 220

Leu Ala Gln Gln Gln Asp Lys His Arg Ser Asp Ile Lys Thr Leu Ala
225 230 235 240

Lys Thr Ser Ala Ala Asn Thr Asp Arg Ile Ala Lys Asn Lys Ala Asp
245 250 255

Asp Asp Ala Ser Phe Glu Thr Leu Thr Lys Asn Gln Asn Thr Leu Ile
260 265 270

Glu Lys Asp Lys Glu His Asp Lys Leu Ile Thr Ala Asn Lys Thr Ala
275 280 285

Ile Asp Ala Asn Lys Ala Ser Ala Asp Thr Lys Phe Ala Ala Thr Ala
290 295 300

Asp Ala Phe Thr Lys Asn Gly Asn Ala Ile Thr Lys Asn Ala Lys Ser
305 310 315 320

Ile Thr Asp Leu Gly Thr Lys Val Asp Gly Phe Asp Ser Arg Val Thr
325 330 335

Ala Leu Asp Thr
340

<210> 10

<211> 157

<212> PRT

<213> protein fragment from Moraxella catarrhalis (UspA2 302-458) or synthetic peptide

<400> 10

Ile Gln Asp Leu Ala Thr Tyr Asn Glu Leu Gln Asp Gln Tyr Ala Gln
1 5 10 15

```
Lys Gln Thr Glu Ala Ile Asp Ala Leu Asn Lys Ala Ser Ser Glu Asn
            20                  25                  30

Thr Gln Asn Ile Glu Asp Leu Ala Ala Tyr Asn Glu Leu Gln Asp Ala
        35                  40                  45

Tyr Ala Lys Gln Gln Thr Glu Ala Ile Asp Ala Leu Asn Lys Ala Ser
    50                  55                  60

Ser Glu Asn Thr Gln Asn Ile Glu Asp Leu Ala Ala Tyr Asn Glu Leu
65                  70                  75                  80

Gln Asp Ala Tyr Ala Lys Gln Gln Ala Glu Ala Ile Asp Ala Leu Asn
                85                  90                  95

Lys Ala Ser Ser Glu Asn Thr Gln Asn Ile Ala Lys Asn Gln Ala Asp
            100                 105                 110

Ile Ala Asn Asn Ile Thr Asn Ile Tyr Glu Leu Ala Gln Gln Gln Asp
        115                 120                 125

Lys His Arg Ser Asp Ile Lys Thr Leu Ala Lys Thr Ser Ala Ala Asn
    130                 135                 140

Thr Asp Arg Ile Ala Lys Asn Lys Ala Asp Asp Asp Ala
145                 150                 155
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