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Dated this sixteenth day of March 1998

Signature of translator: _____

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New adhesin from Helicobacter pylori

Description

The present invention concerns the new adherence gene alpB from Helicobacter pylori and the polypeptide coded thereby. The gene, the polypeptide and an antibody directed against the polypeptide can be used to diagnose, prevent and treat a Helicobacter infection.

The occurrence of spiral bacteria in the human gastric mucous membrane has been known for a long time (Bizzozero, 1893). The fact that these are pathogenic germs was, however, not realised until the successful isolation and culture of this bacterium by Marshall and Warren (Warren and Marshall, 1983; Marshall et al., 1984) from the gastric mucous membrane of a patient with a gastric ulcer (ulcus ventriculi). As the first analyses showed the isolated microorganisms were gram-negative, spiral bacteria with an extremely high motility and the unusual ability of being able to survive in a strongly acidic environment (up to ca. pH 1.5). The germs which were originally denoted Campylobacter pylori were finally classified on the basis of biochemical and morphological characteristics in the newly established genus "Helicobacter" (Goodwin et al., 1989).

The importance of Helicobacter pylori infection and the implications of this discovery already became clear within a few years. Epidemiological investigations by Taylor and Blaser (1991) showed that the H. pylori infection occurs world-wide and that ca. 50 % of the

population are infected with this bacterium, the infection rate being higher in the developing countries than in industrialised countries. Furthermore it was observed that the probability of a chronic H. pylori infection increases drastically with increasing age. Hence the H. pylori infection is among the most frequent chronic bacterial infections of humans.

Today it is known that the infection inevitably leads to the induction of a bacterial gastritis (type B gastritis) in humans. Moreover it is assumed that H. pylori also plays a causal role in the development of gastric and duodenal ulcers (ulcus ventriculi and ulcus duodeni) as well as some forms of gastric carcinoma (adenocarcinoma) (Lee et al., 1993; Solnick and Tompkins, 1993). Even the MALT (mucosa associated lymphoid tissue) lymphomas of the stomach which occur more rarely and are regarded as precursors of B cell tumours of the immune system are also presumably a result of H. pylori infection. An antibacterial treatment of such patients with the successful eradication (total elimination) of H. pylori leads to a healing of gastric ulcers as well as of low grade MALT lymphomas (Sipponen and Hyvärinen, 1993; Isaacson and Spencer, 1993; Stolte and Eidt, 1993).

A sequel of a long-term infection with H. pylori is atrophic gastritis, a degeneration of the mucous, acid or pepsin-producing cells of the stomach epithelium which has to be regarded as a pre-cancerous lesion. According to a statistic of the types of cancer which occurred world-wide most frequently in 1980, gastric carcinoma is in second place but with a declining tendency (Parkin et al., 1988). Two studies have recently shown a statistically significant correlation

between H. pylori infection and the occurrence of gastric carcinoma (intestinal type); both came to the conclusion that ca. 60 % of all gastric carcinomas that occur are probably due to a H. pylori infection (Parsonnet et al., 1991; Nomura et al., 1991). Furthermore investigations by Sipponen (1992) show that in many industrialised countries more than 20 % of infected persons contract an ulcer of the stomach or of the duodenum during their life whereas this risk is negligibly small in persons with a normal gastric mucosa. This means that these frequent gastro-duodenal diseases must be regarded as infectious diseases and treated appropriately (Alper, 1993). A treatment which eliminates a chronic H. pylori infection that is already present leads to a healing of a gastritis, a gastric or duodenal ulcer or a MALT lymphoma. Thus a prophylactic treatment which prevents a H. pylori infection (e.g. immunization) as well as a treatment which eliminates a H. pylori infection that is already present can be used to treat these frequent gastro-duodenal diseases.

Apart from some higher primates, humans were previously the only known natural host for H. pylori. The relatively recent discovery that the domestic cat can also be infected with H. pylori throws new light onto the question of transmission and a possible reservoir for these bacteria outside the human organism. The occasionally successful culture of H. pylori from the faeces of infected persons and the ability of the bacteria to survive for months in water support the hypothesis of a faecal-oral transmission. Also direct oral-oral transmission is regarded as probable on the basis of family studies. The infection usually occurs in childhood within the family, cramped living conditions and a poor standard of hygiene correlating positively

with the frequency of the infection.

After oral uptake the bacteria first reach the extremely acid stomach lumen (pH 1-2). Here the survival of the bacteria is made possible by the production of the enzyme urease which leads to cleavage of the urea that is present and thus to a local neutralization of the acidic pH value in the stomach. By means of chemotactic orientation and flagella-dependent motility the microorganisms then move into the bicarbonate-buffered mucosal layer of the antrum region of the stomach which is in fact their natural habitat. Here they are in a unique ecological niche which, due to the acid barrier, is accessible only to a few competing bacterial species. The microorganisms presumably orientate themselves by means of the pH gradient between the lumen (pH 1-2) and epithelial cell surface (pH 6-7) in order to reach the epithelium. Due to their spiral shape, their motility in viscous mucous, the production of mucous-modifying enzymes and finally their microaerophilic way of living, these germs are optimally adapted to the living conditions in this habitat.

They usually spend their time in the deep crypts of the antrum region where they are protected from external influences such as e.g. acid, pepsin and also from medicines for their eradication such as e.g. antibiotics. Part of the population (ca. 20 %) is closely associated with epithelial cells especially with mucous-producing cells. Under the condition of a gastral metaplasia i.e. the acid-induced formation of gastral epithelium in the duodenum, the metaplastic areas in the duodenum are also colonised which creates the prerequisites for the development of a duodenal ulcer (ulcus duodeni). A complete excretion of the

Helicobacter with the shed mucous is probably prevented by their ability to adhere so that the bacteria can persist for years, decades or even for a life time (chronic infection).

Before the existence and the significance of H. pylori for ulcerous diseases was known, these were treated with so-called antacids or H_2-receptor antagonists. These are substances which inhibit the acid secretion of the parietal cells of the stomach. The action of these pharmaceutical agents usually leads to a healing of the ulcers but, since one of the causes of these ulcers i.e. H. pylori infection, is not eliminated by this, in most cases a re-occurrence of the ulceration (relapse) occurs after a short time period.

A further frequently used therapy in ulcerations is bismuth treatment. Various bismuth salts (CBS, BSS) have a bactericidal effect on H. pylori. However, a total eradication of these germs is only achieved in 8 - 32 % of the cases. The treatment apparently leads to a temporary suppression of the germs but after discontinuing the treatment the infection flares up again in most cases. A long-term therapy with high doses leads to an accumulation of the substance in the liver, kidney and nervous system and has considerable neurological side-effects (Malfertheiner, 1994).

Since it has been discovered that gastroduodenal ulcer diseases are infectious diseases, one aim of treatment is to eradicate the pathogens by antibiotics. Monotherapy with various antibiotics (amoxicillin, nitrofuran, furazolidin, erythromycin a.o.) has, however, turned out not to be satisfactory since even in

this case eradication of the germs only occurs in 0 - 15 % of the cases. The most successful treatment is at present achieved by a combination of an acid blocker (Ompeprazol) with an antibiotic (Amoxicillin) which can lead to eradication rates of up to 80 % (Malfertheiner, 1994). However, antibiotic treatment to eliminate H. pylori is not promising as a long-term solution since it has to be assumed that the bacteria will rapidly develop resistance to the antibiotics.

There is therefore a need for new forms of therapy to control a H. pylori infection and in particular for vaccines which are specifically directed against virulence factors of H. pylori. Virulence factors denote the properties of a pathogenic bacterium which enable it to colonise a particular ecological niche in the body of the host and to multiply there despite the immune response and the unspecific defence mechanisms of the host organism. Knowledge about virulence factors therefore aids in the better understanding of the course and mechanisms of an infectious disease. The most important previously examined virulence factors of H. pylori are urease, the flagella, the adhesins and the production of a cytotoxin.

Urease, an enzyme on the surface of the bacteria is composed of two subunits (UreA, 26 kDa, UreB, 66 kDa) which constitute up to 5 % of the entire bacterial protein. Urease cleaves the urea which occurs in small concentrations in gastric juice into ammonia and carbon dioxide. According to the current perception the bacterium surrounds itself with a cloud of ammonia which leads to a local neutralization of the acid of the gastric juice. The extremely high motility of the bacteria can be attributed to the presence of polar

flagella which enable the bacterium to move in the viscous mucous of the gastric mucous membrane and thus to reach the epithelial cell layer. The urease gene cluster (ureA - ureH) as well as the genes for the formation of the flagella (flaA, flaB) were cloned in E. coli and sequenced and isogenic mutants were constructed.

Approximately 50 - 60 % of all isolated H. pylori strains produce an 87 kDa protein, the so-called vacuolising cytotoxin, which induces the formation of cytoplasmic vacuoles in in vitro cell cultures. The vacA gene which codes for the cytotoxin of H. pylori has also been cloned in the meantime and genetically characterized. Furthermore it is presumed that the cytotoxin-producing strains have a higher pathogenic potential than strains which do not produce this toxin. In addition a positive correlation was found between the production of the cytotoxin and the development of gastric ulcers.

Investigations on the adherence of H. pylori to epithelial cell lines in vitro show that the bacteria can bind to many cell lines of different tissues. In contrast H. pylori exhibits a very pronounced species and tissue selective adherence (tropism) in the host organism. Thus the bacteria are found only bound to epithelial cells which are of the gastral type of epithelial cells. This selectivity is explained by a specific interaction between a bacterial adhesin and a specific cellular receptor.

Up to now several potential adhesins of H. pylori have been described and a gene (hpaA) which codes for a so-

called N-acetylneuraminyllactose-binding haemagglutinin was cloned and sequenced (Evans et al., 1993). This is a protein which is supposed to recognize a receptor containing sialic acid on the epithelial cells. The significance of this adhesin for H. pylori infection is, however, controversial. Other potential adhesins are either only characterized by their molecular weight or their receptor binding specificity. These include a 63 kDa protein which appears to be homologous to the exoenzyme S of Pseudomonas aeruginosa an adhesin with ADP-ribosyl-transferase activity. Furthermore it is suspected that there is a yet unidentified adhesin which mediates a specific binding to the Lewis^b blood group antigen of gastric epithelial cells (Falk et al., 1993; Borén et al., 1993).

Infection with H. pylori leads to a chronic inflammatory reaction of the gastric mucosa (gastritis). In addition a specific systemic immune response to H. pylori antigens is induced; the formation of secretory antibodies in the stomach (sIgA) has, however, not yet been indisputably clarified. As a result of the inflammation various immune cells are present in the gastric mucosa and submucosa e.g. polymorphonuclear leucocytes, monocytes, macrophages, lymphocytes and plasma cells (Blaser, 1992). In addition H. pylori activates neutrophils as well as monocytes and macrophages in vitro (Mai et al., 1991). Experiments with specific antibodies and complement show a rapid inactivation of H. pylori by neutrophils in vitro. However, in the in vivo situation these mechanisms do not lead to an inactivation of the pathogenic bacteria. How H. pylori survives for a long period in the host although it activates the aforementioned defence mechanism is unclear.

The host is not able to cope with the H. pylori infection under natural conditions. It was therefore even more surprising that the urease, an essential virulence factor of H. pylori (see above), has great potential as a vaccine (US Patent Application USSN 07/970,996 Urease-based vaccine against Helicobacter Infection).

In the Helicobacter felis / mouse model (H. felis is a Helicobacter species which naturally colonises the stomach of the cat and can also infect the mouse) it was possible to show that oral vaccination of the H. pylori urease or the recombinant urease B subunit (rUreB) can protect mice against H. felis infection (preventive vaccine) and can also eliminate an infection which is already present (therapeutic vaccine) (Michetti et al., 1994; Corthesy-Theulaz et al., Gastroenterol., in the press). A decisive factor in the oral vaccination was the use of adjuvants such as e.g. cholera toxin which among others appears to be important for converting the immune reaction from the production of systemic antibodies to secretory antibodies.

The object of the present invention was to provide new secretory genes from Helicobacter pylori and polypeptides coded thereby which are potential candidates for vaccines.

In the German Patent Application 195 21 312.2 a method for identifying secretory genes from Helicobacter pylori is described wherein a mutant gene bank of H. pylori is set up with the aid of a transposon and this mutant collection is analysed for defects in adherence behaviour e.g. towards human gastric epithelial cells.

In this manner it was possible to identify an adhesin gene denoted alpA and a polypeptide coded by this gene.

While cloning the region flanking alpA located downstream a new adhesin gene from H. pylori denoted alpB having the nucleotide sequence shown in SEQ ID NO.1 and a polypeptide coded by this gene having the amino acid sequence shown in SEQ ID NO. 1 and 2 were identified.

Thus a subject matter of the present invention is a DNA molecule which comprises

(a) the nucleotide sequence shown in SEQ ID NO.1
(b) a nucleotide sequence which corresponds to the sequence according to (a) within the scope of the degeneracy of the genetic code or
(c) a nucleotide sequence which hybridizes with the sequences according to (a) or/and (b) under stringent conditions.

In addition to the nucleotide sequence shown in SEQ ID NO.1 and a nucleotide sequence corresponding to this sequence within the scope of the degeneracy of the genetic code, the present invention also encompasses a DNA sequence which hybridizes with one of these sequences under stringent conditions. The term "hybridization" according to the present invention is used as described by Sambrook et al (Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), 1.101 to 1.104). According to the present invention a hybridization under stringent conditions means that a positive hybridization signal is still observed after washing for 1 hour with 1 x SSC and 0.1 % SDS at 55°C preferably at 62°C and especially preferably at 68°C, in particular for 1 hour in 0.2 x SSC and 0.1 %

SDS at 55°C preferably at 62°C and especially preferably at 68°C. The present invention encompasses a nucleotide sequence hybridizing under such washing conditions with one of the nucleotide sequences shown in SEQ ID NO.1 or with a corresponding nucleotide sequence within the scope of the degeneracy of the genetic code.

The DNA molecule according to the invention preferably codes for a polypeptide having the ability to adhere to human cells in particular to human gastric epithelial cells. In addition it is preferred that the DNA molecule according to the invention has a homology at the nucleotide level of at least 70 %, particularly preferably of at least 80 %, to the nucleotide sequence shown in SEQ ID NO.1. Furthermore it is preferred that the DNA molecule has a length of at least 15 preferably of at least 20 nucleotides.

The DNA sequence of the adhesin gene alpB shown in SEQ ID NO.1 codes for a polypeptide of 518 amino acids. The amino acid sequence of this polypeptide denoted AlpB is shown in SEQ ID NO. 1 and 2. An analysis of the N-terminal region of the amino acid sequence of AlpB suggests that the polypeptide has a classical prokaryotic signal sequence. A comparison at the amino acid level between AlpB and the polypeptide AlpA described in the German Patent Application 195 21 312.2 whose amino acid sequence is shown in SEQ ID NO.3 and 4 shows that 46 % of the entire amino acid sequence is identical in both polypeptides. The C-terminal part (amino acids 341-518) even exhibits 66 % identity.

Defect mutants in the alpB gene which were produced by inserting the transposon TnMax9 at two different

positions on the alpB gene do not bind to tissue sections of gastric epithelial cells. Since a stable expression of AlpA was detected in these alpB mutants, the defect in the alpB gene must be directly responsible for the loss of adherence of the alpB mutant.

There is a functional connection between AlpA and AlpB in that AlpB and AlpA can presumably together form a complex. This complex could be present as a heterodimeric or/and multimeric aggregate which is effective as a functional adhesin. The loss of one subunit whether AlpA or AlpB can already lead to loss of the function of the adhesin complex and thus tc adherence defects of the bacteria.

Therefore a further subject matter of the present invention is a DNA molecule which comprises sequence regions of the alpB gene and of the alpA gene that are fused together. In particular this is a DNA molecule in which the sequence defined above under (a), (b) and (c) is fused with

(d) the nucleotide sequence shown in SEQ ID NO.3
(e) a nucleotide sequence which corresponds to the sequence according to (d) within the scope of the degeneracy of the genetic code or
(f) a nucleotide sequence which hybridizes with the sequences according to (d) or/and (c) under stringent conditions.

A preferred example of such a fused DNA molecule contains one or several sections from each of the adhesin genes alpB (SEQ ID NO.1) and alpA (SEQ ID NO.3). The lengths of these sections is preferably at least 18 nucleotides, particularly preferably at least 30

nucleotides and most preferably at least 60 nucleotides.

A further subject matter of the present invention is a vector which contains at least one copy of a DNA molecule according to the invention. This vector can be any prokaryotic or eukaryotic vector on which the DNA sequence according to the invention is located preferably under the control of an expression signal (promoter, operator, enhancer etc.). Examples of prokaryotic vectors are chromosomal vectors such as for example bacteriophages (e.g. bacteriophage λ) and extrachromosomal vectors such as plasmids, whereby circular plasmid vectors are especially preferred. Suitable prokaryotic vectors are e.g. described by Sambrook et al., Supra, chapters 1 to 4.

On the other hand the vector according to the invention can also be a eukaryotic vector e.g. a yeast vector or a vector suitable for higher cells (e.g. a plasmid vector, viral vector, plant vector). Such vectors are for example described by Sambrook et al., Supra, chapter 16.

Yet a further subject matter of the present invention is a cell which is transformed with a vector according to the invention. In a preferred embodiment the cell is a prokaryotic cell preferably a gram-negative prokaryotic cell particularly preferably an E. coli cell. However, on the other hand the cell according to the invention can also be a eukaryotic cell such as a fungal cell (e.g. yeast), an animal or a plant cell.

The invention also concerns a polypeptide which is coded by a DNA molecule according to the invention. The polypeptide is preferably capable of adhering to human

cells and comprises (a) the amino acid sequence shown in SEQ ID NO.2 or (b) an amino acid sequence that immunologically cross-reacts with the sequence according to (a).

The polypeptide according to the invention preferably has a homology of at least 80 % and most preferably of at least 90 % to the amino acid sequence shown in SEQ ID NO.2.

The polypeptides according to the invention are preferably produced by transforming a cell with a DNA molecule or vector according to the invention, culturing the transformed cell under conditions in which expression of the polypeptide takes place and isolating the polypeptide from the cell or/and the culture supernatant. In this process the polypeptide according to the invention can be obtained as a fusion polypeptide as well as a non-fusion polypeptide.

Yet a further subject matter of the present invention are fusion polypeptides which each contain one or several sections from the polypeptides AlpB (SEQ ID NO.2) and AlpA (SEQ ID NO.4). The lengths of these sections is at least 6, preferably at least 10 and most preferably at least 20 amino acids.

Since the AlpB protein can form a functionally active complex with the AlpA protein disclosed in DE 195 21 312.2, the present invention also concerns a polypeptide complex which contains at least two polypeptide components the first component being coded by the alpB sequence or a sequence derived therefrom and the second component being coded by the alpA sequence or a sequence

derived therefrom in particular by a DNA molecule which comprise

(d) the nucleotide sequence shown in SEQ ID NO.3

(e) a nucleotide sequence which corresponds to the sequence according to (d) within the scope of the degeneracy of the genetic code or

(f) a nucleotide sequence which hybridizes with the sequences according to (d) or/and (e) under stringent conditions.

The second polypeptide i.e. the AlpA component of the complex preferably comprises the amino acid sequence shown in SEQ ID NO.4, an amino acid sequence which is at least 80 % and preferably at least 90 % homologous to this sequence or an amino acid sequence which immunologically cross-reacts with these sequences.

The polypeptide AlpB according to the invention or parts thereof can be used as an immunogen to produce antibodies. The present invention therefore also concerns an antibody which is directed against a polypeptide according to the invention or a complex according to the invention. The antibody is preferably directed against the N-terminus e.g. the first 340 and in particular the first 250 amino acids of the amino acid sequence shown in SEQ ID NO.2.

Yet a further aspect of the present invention concerns a pharmaceutical composition which contains a DNA molecule according to the invention, a polypeptide according to the invention, a polypeptide complex according to the invention or an antibody according to the invention as the active substance optionally together with common pharmaceutical auxiliary substances, diluents, additives

and carriers.

The pharmaceutical composition according to the invention can be used on the one hand to diagnose a Helicobacter pylori infection. The diagnosis at the nucleic acid level is preferably carried out by using hybridization probes which contain a DNA sequence according to the invention that is specific for the alpB gene or by amplification using DNA molecules according to the invention as primers. At the protein level the diagnostics are preferably carried out with the aid of the antibodies according to the invention.

On the other hand the pharmaceutical composition can also be used to prevent or fight a Helicobacter pylori infection. For therapeutic applications the AlpB polypeptide or parts thereof optionally together with the AlpA polypeptide or parts thereof are used to produce an active vaccine or the antibody is used to produce a passive vaccine.

It is intended to further elucidate the invention by the following examples and figures.

Fig. 1 shows a restriction map of the plasmid pMu140 which contains the regulatory region and the 5' end of the alpA gene (SEQ ID NO.3). The alpA gene is inactivated by insertion of the transposon TnMax9 (see triangle labelled TnMax9). When the plasmid is expressed an alpA-ß-lactamase fusion protein is obtained. pMu140 is the original clone from the mutant gene bank from which the adherence-defective H. pylori strain P1-140 was obtained by retransformation

and homologous recombination.

Fig. 2 shows a restriction map of the plasmid pMT5 which contains a part of the alpA gene and the entire alpB gene (SEQ ID NO.1). The origin of replication ori_{fd} and the chloramphenicol transferase gene cat_{GC} from the transposon TnMax 9 were used for the selective cloning back of the mutated alpA gene locus and of the flanking alpB gene region. Res denotes the resolution site of TnMax9 and IR denotes the inverted repeats of the transposon. M13-FP and M12-RP1 denote regions which contain the sequences of M12-FORWARD and REVERSE sequencing primers. The polylinker region between the cloning sites SacI and StuI originates from the plasmid pBluescript II KS.

Fig. 3 shows an amino acid sequence comparison of the adhesins AlpA and AlpB. (A) The sequence comparison was carried out using the GCG program BESTFIT (Devereux et al., 1984) and covers the entire polypeptides AlpA and AlpB. Vertical lines denote identical amino acids; points show conserved amino acid substitutions. The degree of similarity or identity between both sequences is stated at the end of the sequence. (B) A significantly higher degree of identity can be seen in the C-terminal region of both polypeptides (position 341-518) which is presumably responsible for the integration of the proteins into the external bacterial membrane. The C-terminal region of five amino acids which ends with a phenylalanine (F) is also typical which in the case of a secreted protein of a gram-

negative bacterium indicates an insertion into the external membrane (Struyvé et al., 1991).

SEQ ID NO.1 shows the nucleotide sequence of the H. pylori adherence gene alpB and the corresponding amino acid sequence.

SEQ ID NO.2 shows the amino acid sequence of the AlpB adherence polypeptide from H. pylori.

SEQ ID NO.3 shows the nucleotide sequence of the H. pylori adherence gene alpA and the corresponding amino acid sequence.

SEQ ID NO.4 shows the amino acid sequence of the AlpA adherence polypeptide from H. pylori.

Example 1

Construction of a H. pylori plasmid gene bank

A plasmid gene bank of the chromosomal DNA of the H. pylori wild-type strain 69A was set up. For this the chromosomal DNA was isolated from H. pylori by the method of Leying et al. (1992) and partially cleaved with each of the restriction endonucleases Sau3AI and HpaII. Subsequently the DNA fragments were separated on a preparative agarose gel and fragments of 3 - 6 kb were eluted from the gel. These DNA fragments were ligated into the plasmid vector pMin2 which was specially constructed for this purpose and had been cleaved with the restriction enzymes BglII and ClaI, ligated (T4 ligase) and the ligation mixture was transformed into

the E. coli strain E 181 which is a derivative of the strain HB101 (Bayer and Roulland-Dussoix, 1969) containing the lysogenes λphages λCH616 and had already been transformed with the transposon TnMax9. In this process ca. 2400 independent transformants were obtained.

The E. coli strain DH5α containing the minimal vector pMin2 is deposited at the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM)", Mascheroder Weg 1b, D-38124 Braunschweig under the file number 10007. The E. coli strain E 181 containing the transposon derivative pTnMax9 is deposited at the DSM under the file number 10008.

Example 2

Isolation of H. pylori mutants

In order to carry out the transposon mutagenesis 10 transformants were pooled in each case and mutually induced by which means a total of 190 pools each with 10-20 clones were treated further. 191 ampicillin resistant E. coli plasmid clones were isolated from this mutagenesis which carried independently mutated H. pylori genes. These 192 plasmids were isolated from E. coli and used for the retransformation of the H. pylori strain 69A. 135 H. pylori mutants were isolated from these 192 transformations which were presumably mutated in genes which code for secretory proteins. The H. pylori mutant collection was then tested in a screening assay for H. pylori mutants which had lost their ability to bind to KatoIII epithelial cells.

For this the mutants were labelled with FITC and cultured for 1 hour at 37°C together with the epithelial cells. The test for adherence was carried out directly by observation with a fluorescence microscope. 2 mutants were found in this case (No. P1-140 and P1-179a) which showed a greatly reduced adherence.

Both mutants also exhibited no adherence in the second adherence model, the tissue sections of human stomach. The H. pylori wild-type strain as well as all further mutants also showed a strong adherence in this model.

The plasmid pMu140 used to produce the mutant strain P1-140 is shown in fig. 1. The plasmid pMu179a (not shown) was used to produce the mutant strain P1-179a. Independent transformations of both plasmids in H. pylori 69A led to the identified adherence defect which proved that no secondary mutations had occurred in the bacterial chromosome but rather that the TnMax9 insertion in the cloned adhesin gene led to the observed phenotype of the H. pylori mutants. The mapping and sequencing of the genes of the plasmid clones pMu140 and pMu179 inactivated by the transposon TnMax9 showed that both clones were the same gene; the transposon was only inserted at different sites. Since the coded protein is a lipoprotein i.e. a protein which is anchored in the membrane with a lipid anchor, the corresponding gene was denoted AlpA (adherence-associated lipoprotein A). Our data from computer secondary structure predictions of membrane proteins and of certain conserved protein sequences at the C-terminus of the protein (C-terminal phenylalanine; Struyvé et al., 1991) argue in favour of an integral membrane protein incorporated into the outer membrane of the gram-negative bacteria.

Example 3

Identification of the alpB adherence gene

During the genetic characterization of the AlpB gene locus the flanking genomic sequences were cloned in E. coli and sequenced. The cloning of the downstream alpA-flanking region was achieved by back-cloning the TnMax9-insertion from the chromosome of H. pylori. Chromosomal DNA of the mutant P1-179a (see example 2) was cleaved with the restriction endonucleases SacI and StuI, the DNA fragments that were formed were circularised together with a SacI-HincII fragment from the polylinker of the plasmid pBluescript II KS using T4 ligase and transformed into competent E. coli E131 cells which were selected on chloramphenicol.

All transformants obtained should contain the mutated alpA gene and have flanking sequences since the replication and propagation could only take place via the origin of replication ori_{fd} inserted chromosomally by TnMax9 and via the chloramphenicol resistance gene located on TnMax9. One of the recombinant plasmids (Fig. 2) obtained pMT5 was analysed further.

Sequencing of pMT5 and subclones constructed therefrom show that there was the start of a further open reading frame following the alpA gene (67 nucleotides after the stop codon) which code for a polypeptide of 518 amino acids and was denoted alpB (SEQ ID NO.1 and 2). Based on the genetic organisation one can assume an operon and alpA and alpB are presumably transcribed by a single promoter (polycystronic mRNA).

The AlpB gene product has exactly the same number of amino acids as AlpA. An analysis of the N-terminal region of the AlpB polypeptide sequence indicates the presence of a classic prokaryotic signal sequence which indicates a secretory polypeptide. A comparison at the amino acid level between AlpA and AlpB shows a 46 % identity over the entire polypeptide. The C-terminal part (amino acids 341-518) shows a 66 % identity (Fig. 3).

Example 4

Functional connection between the adherence genes AlpA and AlpB

In order to investigate a functional connection between AlpA and AlpB, two TnMax9 transposon insertions were introduced at various positions in the alpB gene (pos. 97 and pos. 1108) and transferred by natural transformation into the genome of H. pylori 69a. The correct insertion of the transposon into the H. pylori chromosome and thus the inactivation of the alpB gene was verified by Southern Blot hybridization.

The resulting alpB defect mutants were analysed for their ability to bind to gastric epithelial cells. It was found that both alpB mutants did not bind to gastric epithelial cells from tissue sections.

The stable expression of AlpA in the alpB mutant could be proven by an immunoblot. This shows that AlpB is directly responsible for the adherence defect of the alpB mutant. As a result of the structure prediction and the high homology in the C-terminal region of both

polypeptides one can assume that AlpB is also present inserted into the external bacterial membrane and may be able to form a complex with AlpA. This complex can be effective as a functional adhesin as a heterodimeric or multimeric aggregate whereas the loss of a subunit, whether AlpA or AlpB, already leads to a loss of function of the adhesin complex and thus may lead to an adherence defect of the bacteria.

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SEQUENCE PROTOCOL

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Max-Planck-Gesellschaft zur Foerderung der Wissenschaften e.V. Berlin
(B) ROAD: Hofgartenstr. 2
(C) CITY: Munich
(E) COUNTRY: Germany
(F) POSTAL CODE: 80539

(ii) NAME OF THE INVENTION: New adhesin from Helicobacter pylori

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER-READABLE FORM:
(A) DATA CARRIER: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, version #1.30 (EPA)

(2) INFORMATION FOR SEQ ID NO.1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1557 base pairs
(B) TYPE: nucleotide
(C) STRAND FORM: both
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: genomic DNA

(vi) INITIAL ORIGIN:
(A) ORGANISM: Helicobacter pylori

(vii) DIRECT ORIGIN:
(B) CLONE(S): alpB

(ix) CHARACTERISTICS:
(A) NAME/KEY: CDS
(B) POSITION: 1..1554

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.1:

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ATG ACA CAA TCT CAA AAA GTA AGA TTC TTA GCC CCT TTA AGC CTA GCG        48
Met Thr Gln Ser Gln Lys Val Arg Phe Leu Ala Pro Leu Ser Leu Ala
 1               5                  10                  15

TTA AGC TTG AGC TTC AAT CCA GTG GGC GCT GAA GAA GAT GGG GGC TTT        96
Leu Ser Leu Ser Phe Asn Pro Val Gly Ala Glu Glu Asp Gly Gly Phe
            20                  25                  30
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ATG ACC TTT GGG TAT GAA TTA GGT CAG GTG GTC CAA CAA GTG AAA AAC        144
Met Thr Phe Gly Tyr Glu Leu Gly Gln Val Val Gln Gln Val Lys Asn
          35                  40                  45

CCG GGT AAA ATC AAA GCC GAA GAA TTA GCC GGC TTG TTA AAC TCT ACC        192
Pro Gly Lys Ile Lys Ala Glu Glu Leu Ala Gly Leu Leu Asn Ser Thr
      50                  55                  60

ACA ACA AAC AAC ACC AAT ATC AAT ATT GCA GGC ACA GGA GGC AAT GTC        240
Thr Thr Asn Asn Thr Asn Ile Asn Ile Ala Gly Thr Gly Gly Asn Val
 65                  70                  75                  80

GCC GGG ACT TTG GGC AAC CTT TTT ATG AAC CAA TTA GGC AAT TTG ATT        288
Ala Gly Thr Leu Gly Asn Leu Phe Met Asn Gln Leu Gly Asn Leu Ile
                 85                  90                  95

GAT TTG TAT CCC ACT TTG AAC ACT AGT AAT ATC ACA CAA TGT GGC ACT        336
Asp Leu Tyr Pro Thr Leu Asn Thr Ser Asn Ile Thr Gln Cys Gly Thr
            100                 105                 110

ACT AAT AGT GGT AGT AGT AGT AGT GGT GGT GGT GCG GCC ACA GCC GCT        384
Thr Asn Ser Gly Ser Ser Ser Ser Gly Gly Gly Ala Ala Thr Ala Ala
        115                 120                 125

GCT ACT ACT AGC AAT AAG CCT TGT TTC CAA GGT AAC CTG GAT CTT TAT        432
Ala Thr Thr Ser Asn Lys Pro Cys Phe Gln Gly Asn Leu Asp Leu Tyr
    130                 135                 140

AGA AAA ATG GTT GAC TCT ATC AAA ACT TTG AGT CAA AAC ATC AGC AAG        480
Arg Lys Met Val Asp Ser Ile Lys Thr Leu Ser Gln Asn Ile Ser Lys
145                 150                 155                 160

AAT ATC TTT CAA GGC AAC AAC AAC ACC ACG AGC CAA AAT CTC TCC AAC        528
Asn Ile Phe Gln Gly Asn Asn Asn Thr Thr Ser Gln Asn Leu Ser Asn
                165                 170                 175

CAG CTC AGT GAG CTT AAC ACC GCT AGC GTT TAT TTG ACT TAC ATG AAC        576
Gln Leu Ser Glu Leu Asn Thr Ala Ser Val Tyr Leu Thr Tyr Met Asn
            180                 185                 190

TCG TTC TTA AAC GCC AAT AAC CAA GCG GGT GGG ATT TTT CAA AAC AAC        624
Ser Phe Leu Asn Ala Asn Asn Gln Ala Gly Gly Ile Phe Gln Asn Asn
        195                 200                 205

ACT AAT CAA GCT TAT GGA AAT GGG GTT ACC GCT CAA CAA ATC GCT TAT        672
Thr Asn Gln Ala Tyr Gly Asn Gly Val Thr Ala Gln Gln Ile Ala Tyr
    210                 215                 220

ATC CTA AAG CAA GCT TCA ATC ACT ATG GGG CCA AGC GGT GAT AGC GGT        720
Ile Leu Lys Gln Ala Ser Ile Thr Met Gly Pro Ser Gly Asp Ser Gly
225                 230                 235                 240

GCT GCC GCA GCG TTT TTG GAT GCC GCT TTA GCG CAA CAT GTT TTC AAC        768
Ala Ala Ala Ala Phe Leu Asp Ala Ala Leu Ala Gln His Val Phe Asn
                245                 250                 255

TCC GCT AAC GCC GGG AAC GAT TTG AGC GCT AAG GAA TTC ACT AGC TTG        816
Ser Ala Asn Ala Gly Asn Asp Leu Ser Ala Lys Glu Phe Thr Ser Leu
            260                 265                 270

GTG CAA AAT ATC GTC AAT AAT TCT CAA AAC GCT TTA ACG CTA GCC AAC        864
Val Gln Asn Ile Val Asn Asn Ser Gln Asn Ala Leu Thr Leu Ala Asn
        275                 280                 285
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AAC GCT AAC ATC AGC AAT TCA ACA GGC TAT CAA GTG AGC TAT GGC GGG          912
Asn Ala Asn Ile Ser Asn Ser Thr Gly Tyr Gln Val Ser Tyr Gly Gly
    290                 295                 300

AAT ATT GAT CAA GCG CGA TCT ACC CAA CTA TTA AAC AAC ACC ACA AAC          960
Asn Ile Asp Gln Ala Arg Ser Thr Gln Leu Leu Asn Asn Thr Thr Asn
305                 310                 315                 320

ACT TTG GCT AAA GTT AGC GCT TTG AAT AAC GAG CTT AAA GCT AAC CCA         1008
Thr Leu Ala Lys Val Ser Ala Leu Asn Asn Glu Leu Lys Ala Asn Pro
                325                 330                 335

TGG CTT GGG AAT TTT GCC GCC GGT AAC AGC TCT CAA GTG AAT GCG TTT         1056
Trp Leu Gly Asn Phe Ala Ala Gly Asn Ser Ser Gln Val Asn Ala Phe
            340                 345                 350

AAC GGG TTT ATC ACT AAA ATC GGT TAC AAG CAA TTC TTT GGG GAA AAC         1104
Asn Gly Phe Ile Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Glu Asn
        355                 360                 365

AAG AAT GTG GGC TTA CGC TAC TAC GGC TTC TTC AGC TAT AAC GGC GCG         1152
Lys Asn Val Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala
    370                 375                 380

GGC GTG GGT AAT GGC CCT ACT TAC AAT CAA GTC AAT TTG CTC ACT TAT         1200
Gly Val Gly Asn Gly Pro Thr Tyr Asn Gln Val Asn Leu Leu Thr Tyr
385                 390                 395                 400

GGG GTG GGG ACT GAT GTG CTT TAC AAT GTG TTT AGC CGC TCT TTT GGT         1248
Gly Val Gly Thr Asp Val Leu Tyr Asn Val Phe Ser Arg Ser Phe Gly
                405                 410                 415

AGT AGG AGT CTT AAT GCG GGC TTC TTT GGG GGG ATC CAA CTC GCA GGG         1296
Ser Arg Ser Leu Asn Ala Gly Phe Phe Gly Gly Ile Gln Leu Ala Gly
            420                 425                 430

GAT ACT TAC ATC AGC ACG CTA AGA AAC AGC TCT CAG CTT GCG AGC AGA         1344
Asp Thr Tyr Ile Ser Thr Leu Arg Asn Ser Ser Gln Leu Ala Ser Arg
        435                 440                 445

CCT ACA GCG ACG AAA TTC CAA TTC TTG TTT GAT GTG GGC TTA CGC ATG         1392
Pro Thr Ala Thr Lys Phe Gln Phe Leu Phe Asp Val Gly Leu Arg Met
    450                 455                 460

AAC TTT GGT ATC TTG AAA AAA GAC TTG AAA AGC CAT AAC CAG CAT TCT         1440
Asn Phe Gly Ile Leu Lys Lys Asp Leu Lys Ser His Asn Gln His Ser
465                 470                 475                 480

ATA GAA ATC GGT GTG CAA ATC CCT ACG ATT TAC AAC ACT TAC TAT AAA         1488
Ile Glu Ile Gly Val Gln Ile Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys
                485                 490                 495

GCT GGC GGT GCT GAA GTG AAA TAC TTC CGC CCT TAT AGC GTG TAT TGG         1536
Ala Gly Gly Ala Glu Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp
            500                 505                 510

GTC TAT GGC TAC GCC TTC TAA                                            1557
Val Tyr Gly Tyr Ala Phe
        515
```

(2) INFORMATION FOR SEQ ID NO.2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 518 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO.2:

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Met Thr Gln Ser Gln Lys Val Arg Phe Leu Ala Pro Leu Ser Leu Ala
 1               5                  10                  15

Leu Ser Leu Ser Phe Asn Pro Val Gly Ala Glu Glu Asp Gly Gly Phe
            20                  25                  30

Met Thr Phe Gly Tyr Glu Leu Gly Gln Val Val Gln Gln Val Lys Asn
        35                  40                  45

Pro Gly Lys Ile Lys Ala Glu Glu Leu Ala Gly Leu Leu Asn Ser Thr
    50                  55                  60

Thr Thr Asn Asn Thr Asn Ile Asn Ile Ala Gly Thr Gly Gly Asn Val
65                  70                  75                  80

Ala Gly Thr Leu Gly Asn Leu Phe Met Asn Gln Leu Gly Asn Leu Ile
                85                  90                  95

Asp Leu Tyr Pro Thr Leu Asn Thr Ser Asn Ile Thr Gln Cys Gly Thr
            100                 105                 110

Thr Asn Ser Gly Ser Ser Ser Ser Gly Gly Gly Ala Ala Thr Ala Ala
        115                 120                 125

Ala Thr Thr Ser Asn Lys Pro Cys Phe Gln Gly Asn Leu Asp Leu Tyr
    130                 135                 140

Arg Lys Met Val Asp Ser Ile Lys Thr Leu Ser Gln Asn Ile Ser Lys
145                 150                 155                 160

Asn Ile Phe Gln Gly Asn Asn Asn Thr Thr Ser Gln Asn Leu Ser Asn
                165                 170                 175

Gln Leu Ser Glu Leu Asn Thr Ala Ser Val Tyr Leu Thr Tyr Met Asn
            180                 185                 190

Ser Phe Leu Asn Ala Asn Asn Gln Ala Gly Gly Ile Phe Gln Asn Asn
        195                 200                 205

Thr Asn Gln Ala Tyr Gly Asn Gly Val Thr Ala Gln Gln Ile Ala Tyr
    210                 215                 220

Ile Leu Lys Gln Ala Ser Ile Thr Met Gly Pro Ser Gly Asp Ser Gly
225                 230                 235                 240

Ala Ala Ala Ala Phe Leu Asp Ala Ala Leu Ala Gln His Val Phe Asn
                245                 250                 255

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

Val Gln Asn Ile Val Asn Asn Ser Gln Asn Ala Leu Thr Leu Ala Asn
        275                 280                 285
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Asn Ala Asn Ile Ser Asn Ser Thr Gly Tyr Gln Val Ser Tyr Gly Gly
290 295 300

Asn Ile Asp Gln Ala Arg Ser Thr Gln Leu Leu Asn Asn Thr Thr Asn
305 310 315 320

Thr Leu Ala Lys Val Ser Ala Leu Asn Asn Glu Leu Lys Ala Asn Pro
325 330 335

Trp Leu Gly Asn Phe Ala Ala Gly Asn Ser Ser Gln Val Asn Ala Phe
340 345 350

Asn Gly Phe Ile Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Glu Asn
355 360 365

Lys Asn Val Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala
370 375 380

Gly Val Gly Asn Gly Pro Thr Tyr Asn Gln Val Asn Leu Leu Thr Tyr
385 390 395 400

Gly Val Gly Thr Asp Val Leu Tyr Asn Val Phe Ser Arg Ser Phe Gly
405 410 415

Ser Arg Ser Leu Asn Ala Gly Phe Phe Gly Gly Ile Gln Leu Ala Gly
420 425 430

Asp Thr Tyr Ile Ser Thr Leu Arg Asn Ser Ser Gln Leu Ala Ser Arg
435 440 445

Pro Thr Ala Thr Lys Phe Gln Phe Leu Phe Asp Val Gly Leu Arg Met
450 455 460

Asn Phe Gly Ile Leu Lys Lys Asp Leu Lys Ser His Asn Gln His Ser
465 470 475 480

Ile Glu Ile Gly Val Gln Ile Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys
485 490 495

Ala Gly Gly Ala Glu Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp
500 505 510

Val Tyr Gly Tyr Ala Phe
515

(2) INFORMATION FOR SEQ ID NO.3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1557 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: genomic DNA

(vi) INITIAL ORIGIN:
(A) ORGANISM: Helicobacter pylori

(vii) DIRECT ORIGIN:
(B) CLONE(S): alpA

(ix) CHARACTERISTICS:
(A) NAME/KEY: CDS
(B) POSITION: 1..1554

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

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ATG ATA AAA AAG AAT AGA ACG CTG TTT CTT AGT CTA GCC CTT TGC GCT          48
Met Ile Lys Lys Asn Arg Thr Leu Phe Leu Ser Leu Ala Leu Cys Ala
    520                 525                 530

AGC ATA AGT TAT GCC GAA GAT GAT GGA GGG TTT TTC ACC GTC GGT TAT          96
Ser Ile Ser Tyr Ala Glu Asp Asp Gly Gly Phe Phe Thr Val Gly Tyr
535                 540                 545                 550

CAG CTC GGG CAA GTC ATG CAA GAT GTC CAA AAC CCA GGC GGC GCT AAA         144
Gln Leu Gly Gln Val Met Gln Asp Val Gln Asn Pro Gly Gly Ala Lys
                555                 560                 565

AGC GAC GAA CTC GCC AGA GAG CTT AAC GCT GAT GTA ACG AAC AAC ATT         192
Ser Asp Glu Leu Ala Arg Glu Leu Asn Ala Asp Val Thr Asn Asn Ile
            570                 575                 580

TTA AAC AAC AAC ACC GGA GGC AAC ATC GCA GGG GCG TTG AGT AAC GCT         240
Leu Asn Asn Asn Thr Gly Gly Asn Ile Ala Gly Ala Leu Ser Asn Ala
        585                 590                 595

TTC TCC CAA TAC CTT TAT TCG CTT TTA GGG GCT TAC CCC ACA AAA CTC         288
Phe Ser Gln Tyr Leu Tyr Ser Leu Leu Gly Ala Tyr Pro Thr Lys Leu
    600                 605                 610

AAT GGT AGC GAT GTG TCT GCG AAC GCT CTT TTA AGT GGT GCG GTA GGC         336
Asn Gly Ser Asp Val Ser Ala Asn Ala Leu Leu Ser Gly Ala Val Gly
615                 620                 625                 630

TCT GGG ACT TGT GCG GCT GCA GGG ACG GCT GGT GGC ACT TCT CTT AAC         384
Ser Gly Thr Cys Ala Ala Ala Gly Thr Ala Gly Gly Thr Ser Leu Asn
                635                 640                 645

ACT CAA AGC ACT TGC ACC GTT GCG GGC TAT TAC TGG CTC CCT AGC TTG         432
Thr Gln Ser Thr Cys Thr Val Ala Gly Tyr Tyr Trp Leu Pro Ser Leu
            650                 655                 660

ACT GAC AGG ATT TTA AGC ACG ATC GGC AGC CAG ACT AAC TAC GGC ACG         480
Thr Asp Arg Ile Leu Ser Thr Ile Gly Ser Gln Thr Asn Tyr Gly Thr
        665                 670                 675

AAC ACC AAT TTC CCC AAC ATG CAA CAA CAG CTC ACC TAC TTG AAT GCG         528
Asn Thr Asn Phe Pro Asn Met Gln Gln Gln Leu Thr Tyr Leu Asn Ala
    680                 685                 690

GGG AAT GTG TTT TTT AAT GCG ATG AAT AAG GCT TTA GAG AAT AAG AAT         576
Gly Asn Val Phe Phe Asn Ala Met Asn Lys Ala Leu Glu Asn Lys Asn
695                 700                 705                 710

GGA ACT AGT AGT GCT AGT GGA ACT AGT GGT GCG ACT GGT TCA GAT GGT         624
Gly Thr Ser Ser Ala Ser Gly Thr Ser Gly Ala Thr Gly Ser Asp Gly
                715                 720                 725

CAA ACT TAC TCC ACA CAA GCT ATC CAA TAC CTT CAA GGC CAA CAA AAT         672
Gln Thr Tyr Ser Thr Gln Ala Ile Gln Tyr Leu Gln Gly Gln Gln Asn
            730                 735                 740
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ATC TTA AAT AAC GCA GCG AAC TTG CTC AAG CAA GAT GAA TTG CTC TTA          720
Ile Leu Asn Asn Ala Ala Asn Leu Leu Lys Gln Asp Glu Leu Leu Leu
        745                 750                 755

GAA GCT TTC AAC TCT GCC GTA GCC GCC AAC ATT GGG AAT AAG GAA TTC          768
Glu Ala Phe Asn Ser Ala Val Ala Ala Asn Ile Gly Asn Lys Glu Phe
    760                 765                 770

AAT TCA GCC GCT TTT ACA GGT TTG GTG CAA GGC ATT ATT GAT CAA TCT          816
Asn Ser Ala Ala Phe Thr Gly Leu Val Gln Gly Ile Ile Asp Gln Ser
775                 780                 785                 790

CAA GCG GTT TAT AAC GAG CTC ACT AAA AAC ACC ATT AGC GGG AGT GCG          864
Gln Ala Val Tyr Asn Glu Leu Thr Lys Asn Thr Ile Ser Gly Ser Ala
                795                 800                 805

GTT ATT AGC GCT GGG ATA AAC TCC AAC CAA GCT AAC GCT GTG CAA GGG          912
Val Ile Ser Ala Gly Ile Asn Ser Asn Gln Ala Asn Ala Val Gln Gly
            810                 815                 820

CGC GCT AGT CAG CTC CCT AAC GCT CTT TAT AAC GCG CAA GTA ACT TTG          960
Arg Ala Ser Gln Leu Pro Asn Ala Leu Tyr Asn Ala Gln Val Thr Leu
        825                 830                 835

GAT AAA ATC AAT GCG CTC AAT AAT CAA GTG AGA AGC ATG CCT TAC TTG         1008
Asp Lys Ile Asn Ala Leu Asn Asn Gln Val Arg Ser Met Pro Tyr Leu
    840                 845                 850

CCC CAA TTC AGA GCC GGG AAC AGC CGT TCA ACG AAT ATT TTA AAC GGG         1056
Pro Gln Phe Arg Ala Gly Asn Ser Arg Ser Thr Asn Ile Leu Asn Gly
855                 860                 865                 870

TTT TAC ACC AAA ATA GGC TAT AAG CAA TTC TTC GGG AAG AAA AGG AAT         1104
Phe Tyr Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Lys Lys Arg Asn
                875                 880                 885

ATC GGT TTG CGC TAT TAT GGT TTC TTT TCT TAT AAC GGA GCG AGC GTG         1152
Ile Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala Ser Val
            890                 895                 900

GGC TTT AGA TCC ACT CAA AAT AAT GTA GGG TTA TAC ACT TAT GGG GTG         1200
Gly Phe Arg Ser Thr Gln Asn Asn Val Gly Leu Tyr Thr Tyr Gly Val
        905                 910                 915

GGG ACT GAT GTG TTG TAT AAC ATC TTT AGC CGC TCC TAT CAA AAC CGC         1248
Gly Thr Asp Val Leu Tyr Asn Ile Phe Ser Arg Ser Tyr Gln Asn Arg
    920                 925                 930

TCT GTG GAT ATG GGC TTT TTT AGC GGT ATC CAA TTA GCC GGT GAG ACC         1296
Ser Val Asp Met Gly Phe Phe Ser Gly Ile Gln Leu Ala Gly Glu Thr
935                 940                 945                 950

TTC CAA TCC ACG CTC AGA GAT GAC CCC AAT GTG AAA TTG CAT GGG AAA         1344
Phe Gln Ser Thr Leu Arg Asp Asp Pro Asn Val Lys Leu His Gly Lys
                955                 960                 965

ATC AAT AAC ACG CAC TTC CAG TTC CTC TTT GAC TTC GGT ATG AGG ATG         1392
Ile Asn Asn Thr His Phe Gln Phe Leu Phe Asp Phe Gly Met Arg Met
            970                 975                 980

AAC TTC GGT AAG TTG GAC GGG AAA TCC AAC CGC CAC AAC CAG CAC ACG         1440
Asn Phe Gly Lys Leu Asp Gly Lys Ser Asn Arg His Asn Gln His Thr
        985                 990                 995
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```
GTG GAA TTT GGC GTA GTG GTG CCT ACG ATT TAT AAC ACT TAT TAC AAA      1488
Val Glu Phe Gly Val Val Val Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys
    1000                1005                1010

TCA GCA GGG ACT ACC GTG AAG TAT TTC CGT CCT TAT AGC GTT TAT TGG      1536
Ser Ala Gly Thr Thr Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp
1015                1020                1025                1030

TCT TAT GGG TAT TCA TTC TAA                                         1557
Ser Tyr Gly Tyr Ser Phe
                1035
```

(2) INFORMATION FOR SEQ ID NO.4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 518 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO.4:

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Met Ile Lys Lys Asn Arg Thr Leu Phe Leu Ser Leu Ala Leu Cys Ala
 1               5                  10                  15

Ser Ile Ser Tyr Ala Glu Asp Asp Gly Gly Phe Phe Thr Val Gly Tyr
            20                  25                  30

Gln Leu Gly Gln Val Met Gln Asp Val Gln Asn Pro Gly Gly Ala Lys
        35                  40                  45

Ser Asp Glu Leu Ala Arg Glu Leu Asn Ala Asp Val Thr Asn Asn Ile
    50                  55                  60

Leu Asn Asn Asn Thr Gly Gly Asn Ile Ala Gly Ala Leu Ser Asn Ala
65                  70                  75                  80

Phe Ser Gln Tyr Leu Tyr Ser Leu Leu Gly Ala Tyr Pro Thr Lys Leu
                85                  90                  95

Asn Gly Ser Asp Val Ser Ala Asn Ala Leu Leu Ser Gly Ala Val Gly
            100                 105                 110

Ser Gly Thr Cys Ala Ala Ala Gly Thr Ala Gly Gly Thr Ser Leu Asn
        115                 120                 125

Thr Gln Ser Thr Cys Thr Val Ala Gly Tyr Tyr Trp Leu Pro Ser Leu
    130                 135                 140

Thr Asp Arg Ile Leu Ser Thr Ile Gly Ser Gln Thr Asn Tyr Gly Thr
145                 150                 155                 160

Asn Thr Asn Phe Pro Asn Met Gln Gln Gln Leu Thr Tyr Leu Asn Ala
                165                 170                 175

Gly Asn Val Phe Phe Asn Ala Met Asn Lys Ala Leu Glu Asn Lys Asn
            180                 185                 190

Gly Thr Ser Ser Ala Ser Gly Thr Ser Gly Ala Thr Gly Ser Asp Gly
        195                 200                 205

Gln Thr Tyr Ser Thr Gln Ala Ile Gln Tyr Leu Gln Gly Gln Gln Asn
    210                 215                 220
```

Ile Leu Asn Asn Ala Ala Asn Leu Leu Lys Gln Asp Glu Leu Leu Leu
225 230 235 240

Glu Ala Phe Asn Ser Ala Val Ala Ala Asn Ile Gly Asn Lys Glu Phe
245 250 255

Asn Ser Ala Ala Phe Thr Gly Leu Val Gln Gly Ile Ile Asp Gln Ser
260 265 270

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

Val Ile Ser Ala Gly Ile Asn Ser Asn Gln Ala Asn Ala Val Gln Gly
290 295 300

Arg Ala Ser Gln Leu Pro Asn Ala Leu Tyr Asn Ala Gln Val Thr Leu
305 310 315 320

Asp Lys Ile Asn Ala Leu Asn Asn Gln Val Arg Ser Met Pro Tyr Leu
325 330 335

Pro Gln Phe Arg Ala Gly Asn Ser Arg Ser Thr Asn Ile Leu Asn Gly
340 345 350

Phe Tyr Thr Lys Ile Gly Tyr Lys Gln Phe Phe Gly Lys Lys Arg Asn
355 360 365

Ile Gly Leu Arg Tyr Tyr Gly Phe Phe Ser Tyr Asn Gly Ala Ser Val
370 375 380

Gly Phe Arg Ser Thr Gln Asn Asn Val Gly Leu Tyr Thr Tyr Gly Val
385 390 395 400

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

Ser Val Asp Met Gly Phe Phe Ser Gly Ile Gln Leu Ala Gly Glu Thr
420 425 430

Phe Gln Ser Thr Leu Arg Asp Asp Pro Asn Val Lys Leu His Gly Lys
435 440 445

Ile Asn Asn Thr His Phe Gln Phe Leu Phe Asp Phe Gly Met Arg Met
450 455 460

Asn Phe Gly Lys Leu Asp Gly Lys Ser Asn Arg His Asn Gln His Thr
465 470 475 480

Val Glu Phe Gly Val Val Val Pro Thr Ile Tyr Asn Thr Tyr Tyr Lys
485 490 495

Ser Ala Gly Thr Thr Val Lys Tyr Phe Arg Pro Tyr Ser Val Tyr Trp
500 505 510

Ser Tyr Gly Tyr Ser Phe
515

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Max-Planck-Institut
für Biologie
Spemannstr. 34

72076 Tübingen

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
DH5α (pMin2)	DSM 10007

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
(X) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 1995-05-26 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSM-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address: Mascheroder Weg 1b D-38124 Braunschweig	Date: 1995-05-31

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSM-BP/4 (sole sheet) 07/94

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Max-Planck-Institut
für Biologie
Spemannstr. 34

72076 Tübingen

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Max-Planck-Institut für Biologie Address: Spemannstr. 34 72076 Tübingen	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 10007 Date of the deposit or the transfer[1]: 1995-05-26

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 1995-05-29 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSM-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Mascheroder Weg 1b D-38124 Braunschweig	Date: 1995-05-31

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Max-Planck-Institut
für Biologie
Spemannstr. 34

72076 Tübingen

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
E181 (pTnMax9)	DSM 10008

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
(X) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 1995-05-26 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSM-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address: Mascheroder Weg 1b D-38124 Braunschweig	Date: 1995-05-31

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Max-Planck-Institut
für Biologie
Spemannstr. 34

72076 Tübingen

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Max-Planck-Institut für Biologie Address: Spemannstr. 34 72076 Tübingen	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 10008 Date of the deposit or the transfer[1]: 1995-05-26

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 1995-05-29 [1].
On that date, the said microorganism was

(X)[2] viable

( )[2] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSM-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 1995-05-31

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

Form DSM-BP/9 (sole page) 07/94

Claims

1. DNA molecule,
wherein
it comprises
(a) the nucleotide sequence shown in SEQ ID NO.1
(b) a nucleotide sequence which corresponds to the sequence according to (a) within the scope of the degeneracy of the genetic code or
(c) a nucleotide sequence which hybridizes with the sequences according to (a) or/and (b) under stringent conditions.

2. DNA molecule as claimed in claim 1,
wherein
at the nucleotide level it has a homology of at least 80 % to the nucleotide sequence shown in SEQ ID NO.1.

3. DNA molecule as claimed in claim 1 or 2,
wherein
it has a length of at least 15 nucleotides.

4. DNA molecule as claimed in one of the claims 1 to 3,
wherein
it codes for a polypeptide with the ability to adhere to human cells.

5. DNA molecule as claimed in one of the claims 1 to 4 which is fused with
(d) the nucleotide sequence shown in SEQ ID NO.3
(e) a nucleotide sequence which corresponds to the

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sequence according to (d) within the scope of the degeneracy of the genetic code or

(f) a nucleotide sequence which hybridizes with the sequences according to (d) or/and (e) under stringent conditions.

6. Vector,
wherein
it contains at least one copy of a DNA molecule as claimed in one of the claims 1 to 5.

7. Cell,
wherein
it is transformed with a vector as claimed in claim 6.

8. Polypeptide,
wherein
it is coded by a DNA molecule as claimed in one of the claims 1 to 5.

9. Polypeptide as claimed in claim 8,
wherein
it comprises
(a) the amino acid sequence shown in SEQ ID NO.2,
(b) an amino acid sequence which is at least 80 % homologous to the sequence from (a)
(c) an amino acid sequence which immunologically cross-reacts with the sequences according to (a) or/and (b).

10. Polypeptide, as claimed in claim 8 or 9
wherein
it is capable of adhering to human cells.

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11. Polypeptide complex,
containing at least two polypeptide components wherein the first component is coded by a DNA molecule as claimed in one of the claims 1 to 4 and wherein the second component is coded by a DNA molecule which comprises

(d) the nucleotide sequence shown in SEQ ID NO.3

(e) a nucleotide sequence which corresponds to the sequence according to (d) within the scope of the degeneracy of the genetic code or

(f) a nucleotide sequence which hybridizes with the sequences according to (d) or/and (e) under stringent conditions.

12. Complex as claimed in claim 11,
wherein
the second component comprises

(a) the amino acid sequence shown in SEQ ID NO.4

(b) an amino acid sequence which is at least 80 % homologous to the sequence (a)

(c) an amino acid sequence which immunologically cross-reacts with the sequences according to (a) or/and (b).

13. Process for the production of a polypeptide as claimed in one of the claims 8 to 10,
wherein
a cell is transformed with a DNA molecule as claimed in one of the claims 1 to 5 or a vector as claimed in claim 6, the transformed cell is cultured under conditions in which an expression of the polypeptide takes place and the polypeptide is isolated from the cell or/and the culture supernatant.

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14. Use of a polypeptide as claimed in one of the claims 8 to 10 as an immunogen to produce antibodies.

15. Antibody against a polypeptide as claimed in one of the claims 8 to 10 or against a complex as claimed in claim 11 or 12.

16. Antibody as claimed in claim 15, wherein it is directed against the N-terminus of the amino acid sequence shown in SEQ ID NO.2.

17. Pharmaceutical composition, wherein it contains a DNA molecule as claimed in one of the claims 1 to 5, a polypeptide as claimed in one of the claims 8 to 10, a polypeptide complex as claimed in claim 11 or 12 or an antibody as claimed in claim 15 or 16 as the active substance, optionally together with common pharmaceutical auxiliary substances, diluents, additives and carriers.

18. Use of a pharmaceutical composition as claimed in claim 17 for the diagnosis of a Helicobacter pylori infection.

19. Use of a pharmaceutical composition as claimed in claim 17 for the production of an agent for the prevention or treatment of a Helicobacter pylori infection.

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