

The present invention concerns nucleotide coding sequences for a urease, such as expressed naturally in Campybolacter pylori and its biological applications, notably for the detection of C.pylori in humans or in the environment.

C.pylori is a Gram-negative bacterium heretofore found exclusively at the mucosa surface of the antral region of the human stomach, and more particularly around the lesions and craters of gastric and duodenal ulcers. 25% of the population are C.pylori carriers: 8% present an ulcerous illness, 9% suffer from non-ulcerous dyspepsia and 8% are asymptomatic carriers.

All the C.pylori isolated and described heretofore possess the following three properties:

The bacteria produce an extremely active urease.

They adhere very strongly to the epithelial cells of the gastric mucosa, this property being manifested in vitro by an extremely strong adhesion to the HeLa cells.

The C.pylori produce and release an enzyme which presents a proteolytic activity leading to the degradation of the mucus and thus a weakening of the natural barrier protecting the gastric mucosa.

5 However, the detection of C.pylori in situ in humans and in the environment, and the study of its pathogenic power - it is considered responsible for active gastritis in humans - is complicated by difficulties in culturing this organism.

10 Its growth is very slow (6 to 7 days on blood-gelosed medium) and must take place in microaerophilic conditions, the oxygen in the air being toxic.

 The search for means of detecting this bacterium has led the inventors to identify the genes in C.
15 pylori responsible for producing urease.

 The determination of the nucleotide sequence of an intragenic fragment has rendered possible the use of a tool as a detection probe for the specific identification of the C. pylori.

20 The invention is thus intended to supply new sequences of nucleotides which are capable of coding, for proteins with urease activity such as those expressed in C. pylori.

 It is also intended to supply fragments of this
25 sequence which may be used as detection probes in C. pylori.

 In addition, the invention aims to supply a protein with urease activity, such as expressed in C.

pylori, of a high degree of purity, and fragments of the said protein.

According to the invention, the sequence of nucleotides is characterized in that it comprises at least a part of a coding sequence for a protein with urease activity such as expressed in C. pylori.

The invention particularly concerns a sequence of nucleotides which is capable of hybridizing with coding genes for a protein with urease activity such as expressed in C. pylori, under the following conditions: 68°C, 6 x SSC (1 x SSC is made up of 0.15M of NaCl and 0.015M of sodium citrate, pH7), in Denhardt medium (1% Ficoll, 1% polyvinylpyrrolidone, 1% bovine serum albumin).

According to another aspect of the invention, the sequence of nucleotides is characterized in that it carries the information required for the production of a protein with urease activity or of its fragments, capable of forming an immunologic complex with antibodies directed against respectively a protein with urease activity such as expressed in C. pylori or fragments of the said protein.

According to the invention, the sequence is also characterized in that it comprises at least a part of a fragment of about 8 kb corresponding to the restriction fragment EcoRI (ClaI, BamHI) - PstI (HindIII).

The enzymatic restriction map of this fragment is represented in Figure 2.

A more specifically preferred sequence comprises at least a part of a fragment of about 4.2 kb ($\pm$ 5%) limited by the end nucleotides respectively situated at a distance of approximately 0.55 kb upstream of site H2, as represented in Figure 2, and of approximately 0.7 kb downstream of site B1, as represented in Figure 2.

The sequences constituted by this fragment carry the information necessary for the expression of a protein with urease activity as expressed naturally in C. Pylori.

The expression of these sequences may intervene in any other micro-organism capable of exploiting to its advantage the information carried by the said sequences, notably in a strain of Campylobacter which is naturally deficient in coding genes for the production of a protein with urease activity (or in a strain of urease⁻ Campylobacter).

According to yet another aspect, the invention concerns a recombinant sequence comprising one of the above-defined sequences, possibly in association with a promoter capable of controlling the transcription of a sequence and a DNA coding sequence for the termination signals of the transcription.

The nucleotide sequences of the invention are characterized in that they are capable of hybridizing with a probe formed from the sequence presenting:

- the following chain (I) of nucleotides:

5 AAGCTTTTAGGGGIGTTAGGGGTTTATCAAAAATCTAAAAAGGCGCTTCTCTCAAGCA
ATTGTGCTACAAACATGAGCAATTTAGGCGCTTAAAGAATACTTAAAATCOCAAGATTTA
GAATTGAAGCATTGCGGATTGGGGATAAGTTTGTGAGCGAATGCATGCGATTGAACAA
GCGAATTTTGGAGGCGAGCAAAGCGGGCATATCATTTTLAGGATTAGCTAAAAAGCGC
GATGCGTTGGGTGCGCTTTGCAAGTGAGCGGTTAGTGTAGAAAGTAAGCTT

10 - or the following chain (II) of nucleotides:

15 AAGCATGATTGATTGCAGAAGGAGTGGTGCGGCGAGTCTTGIGTAATTTAAAGCCAATC
GCAOOGGCTTCAATTTGATOGGCTAAGCTOGCATOGTTAGAAGOGTTAOGCTTAGOCAAG
AAOCTAAATTCATAGAATATCTTCAGGCGCTCTGAGCATOCATTTTAAATTTCTCTG
CCTGGAGTGATAGTAGTOGCATTAGTGOCATCAGCAGGAOOGGTTCAOCCAOCAATCATG
GTGTGTACAOOGCTTOGAAAAGCTGTAGGGATTTGTGGGGTGAAATGAAGTGGATGTGT
GTGTCAATAOCCAGCGGTTAOGATCAAOCCTCAOOGGCTAAGGCTTCAGTAGCAGGA
CCTAOGCTAAGATTGTTTTAAAGOCATCTTGATGTCTTGTAAOOGCTTAAOCAATG
CCAGOGATTTTGOCATCTTAAATAOCCATATOGCTTTATAAATAOOGGTGTAAATCAOG
20 ATTAAAGOGTTAGTGATAATTAAATCAACTCTCTTTGCTAGGGTTGTAGATTGGCTC
ATGCTTCTCTTAGGGGTTTAAOOGCAOOGAATTTAAGCTCTTOGOCATAAATGGTGTA
GTCATGTCTACTTCAGOGATCAAGTCTGATOGGCAATCTCACTTTATOGGCTGTAGTA
GGAOCATACATAGAAACATATCTTTCTGCTAATCTTTTTCATTTCTTACTOCTTAATT
GTTTTACATAGTGTGCATOGCTTTTAGG

- or the following chain (II bis) of nucleotides:

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OCTAAAAGOGATGCAACTATGTAAAAACAATTAAGGAGTAAGAATGAAAAGATTAGC
 AGAAAAGATATGTTTCTATGTATGGTCTACTACAGGOGATAAAGTGAGATTGGGOGAT
 CAGACTTGATOGCTGAAGTAGAACATGACTACACCATTTATGGOGAAGAGCTTAAATTG
 GTGGOGGTAAAAOOOCTAAGAGAAGGCATGAGOCAATCTAACAAOOCTAGCAAAGAAGAG
 TTGGATTTAATTATCACTAAOGCTTTAATOGTGGATTACAOOGGTATTTATAAGOGGAT
 ATTGGTATTAAAGATGGCAAATOGCTGGCATTTGGTAAAGGOGGTAAACAAAGACATGCAA
 GATGGOGTTAAAAACAATCTTAGOGTAGGTCTGCTACTGAAGOCCTTAGOOGGTGAAGGT
 TTGATOGTAAOGGCTGGTGGTATTGACACACACATOCACITCATTTCAOOOCAAACAATC
 OCTACAGCTTTTTOGAAGOGGTGTAAACAACATGATTGGTGGTGGAAOOGGTCTGCTGAT
 GGCATAATGOGACTACTATCACTOCAGGCAGAAGAAATTTAAAATGGATGCTCAGAGOG
 GCTGAAGAATATTCTATGAATTTAGGTTTCTTGGCTAAAGGTAAOGCTTCTAAOGATGOG
 AGCTTAGOOGATCAAATTGAAGOOOGGTGOGATTGGCTTTAAATTTCAOGAAGACTGGGGC
 AOCACCTOCTTCTGCAATCAATCATGGTT

- or the chains of nucleotides which are complementary respectively to the above-mentioned chains (I), (II) and (II bis).

Sequences of nucleotides according to the invention, are characterized in that they comprise the above-defined nucleotide chains or are constituted by at least a part of the said chains.

5 It is of course understood that the bases of the nucleotide sequences may be in a different order from that found in the genes, and/or that the said bases may possibly be substituted, when a probe elaborated from such sequences gives a characteristic and unequivocal
10 answer with respect to the ability to recognize the presence of coding genes for the protein with urease activity of C. Pylori.

The invention also covers any sequence of nucleotides, hybridizable with that of the above-
15 mentioned chains of nucleotides, such as that obtained by reverse enzymatic transcription of the corresponding RNA, or else by chemical synthesis.

The invention also concerns a sequence of nucleotides which corresponds, according to the
20 universal genetic code, to at least a part of the following amino acid sequence (III):

KLIGVLGVYOKSKNALSSQAIVATNMSNLALKEYLKSQLELKHCAIGDKFVSEOMRLNK
ANFGGEQSGHIIIFSDYAKTIGDGLVCAIQVSALVLESKL

5

10

The invention also concerns a sequence of nucleotides which corresponds, according to the universal genetic code, to at least a part of the following amino acid sequence (IV) (coded by the nucleotidic sequence (II bis)):

20

NMTTFMAKSINSVAVKPLREGMSQSNPSKEELDIITNALIVDYTGTYKADIGIKDGK
IAGIGKGGNKMDQGVKNLSVGPATEALAGEGLIVTAGGIDTHIHFI SPQQIPTAFRSG
VTIMIGGGTG PADGINATTTTPGRNLKWLRAAEEYSNLF LAKGNASNDASLADQIE
AGAIGFKIHEDWGTTPSAINHA

25

The invention also concerns recombinant expression and cloning vectors, capable of transforming an appropriate host cell, the said vectors containing at least a part of a sequence of nucleotides as defined above under the control of elements of regulation permitting its expression.

Preferred recombinant vectors include at least a part of the above-mentioned sequence of approximately 4.2 kb.

10 The strains of transformed micro-organisms are also covered by the invention. These strains contain one of the above-defined sequences of nucleotides or a recombinant vector such as previously defined.

Strain E. coli S17-1, registered under number I-15 795 at the Collection Nationale de Culture de Microorganismes (C.N.C.M.) of the Institut Pasteur in PARIS (FRANCE), carries plasmid pILL590 of approximately 16.3 kb comprising the previously mentioned

20 restriction fragment EcoRI (ClaI, BamHI) - PstI (HindIII) of approximately 8 kb.

The invention also concerns a protein presenting a urease activity of the type expressed naturally in C. pylori, as well as the peptide fragments of this protein.

25 In the invention, the protein and its fragments correspond, according to the universal genetic code, to the above-defined sequences of nucleotides, in

particular to at least a part of the sequence of approximately 4.2 kb.

The protein of the invention is also characterized in that it is a protein such as one obtained through
5 the transformation of host cells by means of a recombinant vector as previously defined, culturing, in an appropriate medium, of the transformed host cells and recovery of the protein from these cells or directly from the culture. The invention also concerns
10 the production of urease or of fragments of the said urease by this process.

In the invention, the protein and its fragments, which may also be obtained by chemical synthesis, present advantageously a high degree of purity and are
15 used, according to standard techniques, to form polyclonal antibodies.

Such polyclonal antibodies, as also monoclonal antibodies capable of recognizing specifically the above-mentioned protein and its fragments, are also
20 covered by the invention.

The above-defined sequences of nucleotides are obtained, according to the standard techniques of genetic engineering, by cloning and identification of the genes responsible for the synthesis of a protein
25 with urease activity in C. pylori.

The invention also concerns the biological applications of the sequences of nucleotides, of the

corresponding proteins, and of the monoclonal or polyclonal antibodies.

These applications include the elaboration, from intragenic fragments, of probes for the detection of C. Pylori. This elaboration comprises, in particular, the
5 denaturation of the double-stranded sequences in order to obtain a mono-stranded sequence which may be used as a probe.

The tests performed on such fragments with a view
10 to detecting the possible presence of complementary sequences in various Campylobacteria and in micro-organisms belonging to different genera, have revealed the high degree of specificity of these fragments.

The invention therefore concerns detection probes
15 characterized in that they comprise at least a part of an above-defined sequence of nucleotides.

Any probe which, with regard to its sequence of nucleotides, is distinguished from the previously mentioned probe only in respect of substitutions or
20 alterations of nucleotides not causing modification of its hybridization properties with the genome of C. Pylori, is covered by the invention.

The DNA fragment used as a probe contains a sufficient number of nucleotides to obtain the required
25 specificity and the formation of a stable hybrid.

It is possible to use fragments attaining several kb, but high specificity results are also obtained with

shorter fragments of approximately 25 to 40 nucleotides.

Appropriate probes for this type of detection are advantageously marked by a radio-active element or by any other group allowing its recognition in the hybridized state with the preparation enclosing the DNA to be studied.

According to the standard techniques, these probes are brought into contact with a biological specimen containing the bacteria, or directly with these bacteria or their nucleic acids, in conditions permitting the possible hybridization of a nucleotide sequence of the probe with a complementary sequence, possibly contained in the tested product.

The hybridization method may, for example, be implemented on spots. This method involves, after denaturation of the DNA previously obtained from bacteria originating from antral biopsies, the deposit of an aliquot quantity of this DNA on nitrocellulose membranes, the hybridization of each membrane in usual conditions with the probe, and the detection, in standard fashion, of the hybrid formed.

An hybridization method may also be used, according to the Southern technique. This method involves the electrophoretic separation in agarose gel of the fragments of DNAs engendered after treatment of the DNA by restriction enzymes, transfer after alkaline denaturation on appropriate membranes, and their

hybridization with the probe in usual conditions. It is not always necessary to proceed to the preliminary expression of the DNA; it is enough that the DNA should be rendered accessible to the probe.

5 Detection for the specific identification of the C. pylori may also be carried out by DNA amplification techniques (PCR). These techniques are notably described in US patents 4683202 and 4683195 taken out by Cetus Corporation.

10 To implement these techniques, two primers of 10 to 40 nucleotides are used, advantageously of approximately twenty nucleotides, these primers being included in one of the above-defined sequences of nucleotides or likely to hybridize with a part of one
15 of the above-defined nucleotidic sequences, or of their complementary sequences. When they are hybridized (or linked), these primers are advantageously situated at approximately 200 to 250 nucleotides from the DNA strains to be amplified. One of the sequences is
20 capable of being linked to a nucleotide sequence of one of the DNA fragment strains to be amplified, this sequence being situated at an end of this fragment, for example 5' end; the other sequence is capable of being linked to a nucleotide sequence of the second DNA
25 fragment strain to be amplified; this latter sequence being situated at the end opposite the above-mentioned end of this fragment (which said end is designated by 3' end in the given example).

Preferred primers are included in the nucleotide sequence of restriction fragment H2-H3 in Figure 2, and are approximately 200 to 250 nucleotides apart.

5 In particular, the fragments concerned are those situated respectively at each end of the restriction sequence HindIII-HindIII.

A process of in vitro detection of the possible presence of C. pylori in a biological sample includes the following steps:

- 10 - possibly, the prior amplification of the amount of nucleotide sequences likely to be contained in the sample by bringing this sample into contact with the previously described primers, these primers being likely to be linked respectively to the 5' end of one
15 strain of the said sequence of nucleotides and to the 3' end of the other strain of the said sequence of nucleotides, this contact being followed by a genic amplification step in the presence of a DNA-dependent polymerase and of the four triphosphate nucleosides
20 dATP, dCTP, dGTP and dTTP, with these primers and genic amplification hybridization steps being repeated several times;
- the biological sample in question is brought into contact with a nucleotidic probe according to the
25 invention, in conditions permitting the production of an hybridization complex formed between the probe and the sequence of nucleotides,
- the detection of the hybridization complex.

The invention thus provides the tools necessary for rapid detection of C. pylori, both in situ and in the environment, with a high degree of specificity and without having recourse to culturing.

5 Such detection tools are particularly useful in studying the natural reservoir of these bacteria, as well as the methods of transmission, circulation and contamination. Furthermore, in the in vitro diagnosis performed on the biopsies, the use of these probes
10 makes it possible to gain a considerable amount of time compared to current techniques, which said techniques, moreover, require a technology which can only be applied in specialized services, i.e. bacteriological techniques or the use of electronic microscopy.

15 Reproducible results have been obtained, using the above-defined fragment of approximately 8 kb as intragenic probe.

 Given their specificity with regard to C. pylori, these probes are also tools of considerable interest.

20 In studying the pathogenic power of C. pylori with a view to preventing infection, it is thus possible to study the role played by C. pylori in the development of ulcerous affections, and to identify the genetic determinants which seem to be involved in this
25 organism's pathogenic power, with a view to creating, in vitro, isogenic mutants of C. pylori, i.e. bacteria modified genetically in each of the determinants which seem to play a role in the pathogenesis of the

infection, which said mutants could be tested using animal models.

The invention also concerns the detection of the polymorphism of the coding genes for the urease in C. pylori by means of the above-defined nucleotidic probes and appropriate restriction enzymes. The conditions in which this detection is carried out are, in particular, described in an article by J.F. de Gusella in the Journal of Clinical Investigations (1986), 77, 1723-1726.

The invention also concerns the immunologic applications of the coded protein, particularly for the elaboration of specific antiserum and of polyclonal and monoclonal antibodies. The polyclonal antibodies are formed, according to standard techniques, by injecting the protein into animals, recuperating the antisera and then the antibodies from the sera, e.g. by affinity chromatography.

The monoclonal antibodies are produced in the usual manner by merging the myeloma cells with the cells of female rats previously immunized by means of the proteins of this invention.

In addition, the invention concerns an in vitro detection method for the possible presence of C. pylori in a biological sample in which it is likely to be contained. This procedure is characterized in that it comprises:

- the bringing into contact of the sample with an antibody according to the invention, in conditions allowing the production of an immunologic complex formed between all or part of the protein with urease activity produced by C. pylori and this antibody, and
- the detection of the immunologic complex.

For the implementation of the in vitro detection methods, considered above and based on the use of nucleotidic probes, recourse is advantageously made to equipment or kits comprising:

- possibly, at least a couple of primers according to the invention,
- a determined quantity of a nucleotidic probe according to the invention,
- advantageously, a medium which is appropriate to the formation of a hybridization reaction between the sequence to be detected and the probe.
- advantageously, reagents allowing the detection of hybridization complexes formed between the nucleotide sequence and the probe during the hybridization reaction.

For the implementation of the in vitro detection methods, defined above and based on the use of antibodies, recourse is advantageously made to equipment or kits comprising:

- a determined quantity of an antibody according to the invention,

- advantageously, an appropriate medium for the formation of an immunologic reaction between at least a part of the protein with urease activity produced by a strain of C. pylori and the antibody.

5 - advantageously, reagents permitting the detection of the immunologic complexes formed between at least a part of the protein with urease activity and the antibody during the immunologic reaction.

An in-depth study of the above-defined fragment of
10 4.2 kb has made it possible to situate the coding structure genes for the major polypeptide subunits of the urease of C. pylori.

This study was carried out by constructing mutants in the recombinant plasmids introduced in E. coli and
15 C. jejuni, which are incapable of synthesizing the urease (A. Baskerville, D. Newell (1988), Gut. 29, 465-472). The system of in vitro transcription-translation of E. coli and a system of expression in minicells were used to identify and locate, in the fragment of 4.2 kb
20 of C. pylori, all the coding genes for the urease subunits; two major proteins of 26kDa and 61kDa were identified. Parallel to this, after various deletions within the fragment of 4.2 kb, a system of transfer by conjugation of a strain of E. coli to C. jejuni (of the
25 type expressed in the ensuing detailed description), was used to identify the regions indispensable to the urease activity, and to correlate the deletion of a region with the disappearance of a polypeptide and the

loss of urease activity. In this way, it was determined that the coding genes for the two polypeptides necessary to the expression of the urease activity are situated in the central region of 3.35 kb, downstream of site H3 in Figure 2, and astride B1. Transcription of the coding genes for the polypeptides of 61kDa or 26kDa takes place in the sense EcoRI-PstI. The fragment of 3.35 kb is necessary to, but insufficient for, the expression of the urease activity in C. jejuni. An adjacent region of 0.85kb, situated upstream and apparently not associated with the expression of polypeptides in E. coli, is necessary to the expression of the urease activity in C. jejuni.

Analysis of the deletion mutants by in vitro transcription-translation makes it possible to observe that the two polypeptides of 26kDa and 61kDa are coded by two adjacent DNA fragments.

5 This invention concerns all or part of the following nucleotidic sequence (V):

10

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¹
 ATGAACTCAOOOCAAAAGAGTTAGATAAGTTGATGCTOACTAOGCTGGAGAATTGGCT
 AAAAAOGCAGAGAAAAGGCATTAGCTTAAGCTATGTAGAAGCAGTAGCTTTGATTAGT
 GOOCATATTATGGAGAGAGOGAGAGCTGGTAAAAAGACTGOGGCTGAATTGATGCAAGAA
 GGGOGCACTCTTTTAAAAOCAGATGATGTGATGGATGGOGTGGCAAGCATGATOCATGAA
 GTGGGTATTGAAGOGATGTTTOCTGATGGGACTAACTOGTAAOOGTGCATAOOOCTATT
 GAGGOCATGGTAAATTAGTTTOCTGGTGAGTTGTTCTTAAAAAATGAAGACATCACTATC
 AAOGAAGGCAAAAAAGOOGTTAGOGTGAAAGTTAAAAATGTTGGOGACAGA OOGGTTCAA
 ATOGGCACACACTTOCATTTCCTTGAAGTGAATAGATGOCTAGACTTTGACAGAGAAAAA
 ACTTTGGGTAAAOGCTTAGACATTGOGAGOGGGACAGOGGTAGATTTGAGOCTGGOGAA
 GAAAAATOOGTAGAATTGATTGACATTGGOGGTAAACAGAAGAATCTTTGGATTTAAGCA
 TTGGTTGATAGACAAGCAGACAAGAAAGCAAAAAAATTGCTTTACACAGAGCTAAAGAG
 OGTTGGTTTCATGGOGCTAAAAGOGATGACAACCTATGTAAAAACAATTAAGGAGTAA

717

The invention particularly concerns any nucleotidic sequence corresponding, according to the universal genetic code, to at least a part of the following amino acid sequence (VI) of 26kDa (coded by
5 nucleotidic sequence (V)):

1
MKLTPKELDKLMHYAGELAKKRKEGKLNVEVALISAHIMEEARAGKCTAELMQE
10 GRTLLKPDMDGVASMIHEVGIEAMFPDGTKLVTVHIPIEANGKLVFGELFLKNEDITI
NEGKAVSVKKNWGRPVQIGSHFFFEVNRCLDEDEKTFGKRLDIASGTAVRFEPE
EKSVELIDIGNRRIFGENALVDRQADNESKIALHRAKRGFHGAKSDNYVKTKE
239

15 239 Amino acids
Molecular weight: 26.522 daltons

20

25

The invention also concerns all or part of the following nucleotidic sequence (VII):

1
 5 ATGAAAAGATTAGCAGAAAGATATGTTTCTATGTATGGTCTACTACAGGOGATAA
 GTGAGATTGGGOGATACAGACTTGTATGCTGAAGTAGACATGACTACACATTATGGC
 GAAGAGCTTAAATTGGGTGGGGTAAAOCTAAGAGAGGCATGAGOCATCTAACAC
 OCTAGCAAAGAAGAGTTGGATTATATTATCACTAAOGCTTAAATOGTGGATTACAOGGT
 ATTTATAAAGOGGATATTGGTATTAAAGATGGCAAATOGCTGGCATTTGGTAAAGGOGT
 AACAAAGACATGCAAGATGGOGTTAAAAACAATCTTAGOGTAGGTCTGCTACTGAGOC
 10 TTAGCOGGTGAAGGTTTGTATGTAAOGGCTGGTGGTATTGACACACATOCATTTCATT
 TCAOOCAACAATOOCTACAGCTTTTGCAAGOGGTGTAAACAOCATGATTGGTGGTGG
 AOOGGTCTGCTGATGGCACATAATGOGACTACTATCACTOCAGGCAGAAGAATTAAAA
 TGGATGCTCAGAGOGGCTGAGAAATATCTATGAATTTAGGTTTCTTGGCTAAAGGTAC
 GCTTCTAAOGATGOGAGCTTAGOOGATCAAATTGAAGOOGGTGGATTGGCTTTAAATT
 CAOGAAGACTGGGGCAOACTOCTTCIGCAATCAATCATGOGTTAGATGTTGOGGACAA
 15 TAOGATGTGCAAGTGGCTATOCACACAGACACTTTGAATGAAGOOGGTTGTGTAGAGAC
 ACTATGGCTGCTATTGCTGGAOGCACTATGCACACTTTTOCACACTGAAGGOGCTGGOSG
 GGCAOGCTOCTGATATTATTAAGTAGOOGGTGAACACAACATTCTTTOOGCTTOCACT
 AAOOOCACATOOCTTTCAOOGTGAATACAGAGGCAGGCACATGGACATGCTTATGGTG
 TGOCAOACTTGGATAAAAGCATTAAAGAAGATGTTTCAGTTGGCTGATTCAAGGATOOGC
 OCTCAAOCATTGOGGCTGAAGACACTTTGCATGACATGGGGATTTTCTCAATCAOCAGT
 20 TCTGACTCTCAAGOGATGGGCOGTGTGGGTGAAGTTATCACTAGAACTTGGCAAACAGCT
 GACAAAACAAGAAAGAAATTGGGOOCTTGAAGAAGAAAAGGOGATAAOGACAACCTT
 AGGATCAAAGCTACTTGTCTAAATACACATTAAOOCAGOGATOGCTCATGGGATTAGC
 GAGTATGTAGGTTCAGTAGAAGTGGGCAAAGTGGCTGACTTGGTATTGTGGAGTOCAGCA
 TTCTTTGGOGTGAAAOCAACATGATCATCAAGGOGGATTCATTGOGTTAAGOCAAATG
 GOGATGOGAAOGCTTCTATOOCTAOCOCACAPOGGTTATTACAGAGAAATGTTGGCT
 25 CATCATGGTAAAGCTAAATAOGATGCAACATCACTTTTGTGTCTCAAGOGGCTTATGAC
 AAAGGCATTAAAGAAGAATTAGGACTTGAAGACAAGIGTTGOOGGTAAAAAATTGCAGA
 AATATCACTAAAAAGACATGCAATTCAOGACACTACIGCTCACATTGAAGTCAATOCT
 GAAACTTAOCATGTGTTGGTGGATGGCAAAGAAGTAACTTCTAAOOCAGOCAATAAAGTG
 AGCTTGGOGCAACTCTTAGCATTTCCTAG

The invention particularly concerns any nucleotidic sequence corresponding, according to the universal genetic code, to at least a part of the following amino acid sequence (VIII) of 61kDa (coded by
 5 nucleotidic sequence (VII)):

1
 MKKISRKEYVSMYGPTTGDKVRLGDTDLAEVEHDYTYGEELKFGGGKTLREGM
 SQSNNPSKEELDIITNALIVDYTGMYKADIGIKDGIAGIGKGGNKDMQDGVKNN
 10 LSVGPATEALAGEGLIVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTG PAD
 GTNATTTTPGRRNLKWM LRAAEEYSMNLGFLAKGNASNDASLADQIEAGAIGF
 KIHEDWGTTSPAINHALDVADKYDVQVAHTDTLNEAGCVEDTMAAIAGRTMH
 TFHTEGAGGGHAPDIIKVAGEHNILPASTNPTIPFTVNTEAEHMDMLMVCHLD
 15 KSIKEDVQFADSRIRPQTIAAEDTLHDMGIFSITSSDSQAMGRVGEVITRTWQT
 ADKNKKEFGRLKEEKGDNDNFRIKRYLSKYTINPAIAHGISEYVGSVEVGKVADL
 VLWSPAFFGVKPNMIIKGGFIALSOMGDANASIPTQP VYYREMFAHHGKAKYD
 ANITFVSQAAYDKGIKEELGLERQVLPVKNCRNITKKDMQFNDTTAHIEVNPETY
 20 HVFVDGKEVTSKPANKVSLAQLFSIF
 569

569 Amino acids

Molecular weight: 61 729 daltons

Comparison of amino acid sequence VIII with that of the jack bean urease, notably described in the European Journal of Biochemistry 175, 151-165 (1988), unexpectedly reveals a high level of amino acid homologies. This structure conservation has enabled the inventors to deduce the active site of the urease subunit of 61kDa which is comprised, in whole or in part, in the polypeptide sequence limited by the amino acids situated at positions 206 and 338 of the above-described sequence VIII.

The invention also concerns all or part of nucleotidic sequence IX, constituted successively of sequence V and sequence VII.

The invention also relates to any nucleotidic sequence corresponding, according to the universal genetic code, to at least a part of the amino acid sequence (X) of 87kDa, constituted successively of sequence VI and sequence VIII.

The invention also concerns probes for detecting the possible presence of C. pylori in a biological sample characterized in that they comprise at least a part of an above-defined sequence of nucleotides.

It is of course understood that the bases of the sequences of nucleotides under consideration may be in an order differing from that found in the genes, and/or that these bases may, if required, _____ be substituted, when a probe elaborated from such sequences gives a characteristic and unequivocal

response regarding the ability to recognize the presence of coding genes for protein with urease activity of C. pylori.

5 The invention also covers any sequence of nucleotides hybridizable with that of the above-mentioned chains of nucleotides, such as obtained by reverse enzymatic transcription of the corresponding RNA or by chemical synthesis.

10 The invention also covers any probe which, with regard to its sequence of nucleotides, is distinguished from that previously mentioned only by substitutions and alterations not causing any modification of its hybridization properties with the genome of C. pylori.

15 The above-mentioned probes may be used in vitro processes for diagnosing the possible presence of C. pylori in a biological sample^o such as described above.

20 The invention also concerns nucleotidic primers of approximately 10 to approximately 40 nucleotides, these primers being included in one of the above-defined nucleotidic sequences V or VII (or in one of the sequences derived from the said sequences V and VII), or being likely to hybridize with a part of the above-mentioned nucleotidic sequences or with their
25 complementary sequences (particularly in the above-mentioned conditions of hybridization).

The invention also concerns the use of these primers in the above-mentioned in vitro diagnosis

methods, for the preliminary amplification of the quantity of nucleotides of C. pylori likely to be contained in the biological specimen according to the above-described specimen.

5 The invention also relates to the recombinant vectors of expression and cloning, capable of transforming an appropriate host cell containing all or part of a nucleotidic sequence which is identical to, or derived from, the above-mentioned nucleotidic
10 sequences V, VII and IX.

 The invention also concerns a protein production process corresponding to all or part of the above-mentioned sequences VI, VIII and X, the said process comprising the transformation of appropriate host cells
15 (particularly those described above) by means of the above-mentioned vectors, the recovery of the proteins thus produced followed, in certain cases, by a purification step of the said proteins.

 The above-defined protein production process may,
20 if required, comprise a preliminary amplification step of the coding gene for the protein whose production is sought, this step being carried out with primer couples of the invention in accordance with the above-described process.

25 The invention also concerns the polypeptides (or proteins) coded by the above-described nucleotidic sequences. The invention particularly concerns the above-described amino acid sequence X of 87kDa,

sequence VI of 26kDa and sequence VIII of 61kDa, and their fragments.

The invention also concerns the polyclonal or monoclonal antibodies directed against all or part of the above-described polypeptides and likely to form an immunologic complex with them.

In particular, the invention concerns the antibodies, notably monoclonal, obtained by means of a peptide sequence of at least approximately 10 to 15 amino acids of sequence VI or of sequence VIII.

Preferred monoclonal antibodies of the invention are directed against all or part of the sequence limited by the amino acids situated at positions 206 and 338 of sequence VIII, more especially those obtained by means of a peptide sequence of at least approximately 10 to 15 amino acids originating from the sequence limited by the amino acids situated at positions 206 and 338 of sequence VIII.

The invention also relates to the use of the above-mentioned antibodies to implement the in vitro methods and the in vitro kits as described above, for diagnosing the infection of an individual by C. pylori.

The invention also concerns immunogenic compositions comprising all or part of the above-described polypeptides, and in particular all or part of a polypeptide sequence limited by the amino acids situated at positions 206 and 338 of sequence VIII, in association with a pharmaceutically acceptable vehicle.

Such immunogenic compositions may be used as vaccine compositions in preventing the infection of an individual by C. pylori.

5 The invention also concerns pharmaceutical compositions comprising one (or several) antibodies such as described above, and in particular, antibodies likely to form an immunologic complex with all or part of the peptide sequence limited by the amino acids situated at positions 206 and 338 of sequence VIII, in
10 association with a pharmaceutically acceptable vehicle.

Such pharmaceutical compositions, according to the invention, may be used in the treatment of pathologies linked to the infection of an individual by C. pylori, notably gastritis, and gastric and duodenal ulcers.

15 Other advantages and characteristics are detailed in the following description relating to the cloning of genes responsible for the production of the protein with urease activity in C. pylori, the sequencing of the region associated with the expression of the
20 protein with urease activity and the specificity of nucleotidic probes for detecting C. pylori.

In this description, reference is made to Figures 1 and 2:

- Figure 1 represents the restriction map of a fragment
25 of PstI-EcoRI of the recombinant cosmid IID2 carrying the information required for the expression of the urease in C. jejuni, and

- Figure 2 represents the restriction map of a fragment of approximately 8 kb of pILL590.

- 1) Construction of a genomic bank in a shuttle cosmid vector; cloning of genes involved in the production of urease in C. pylori.

The chromosome DNA of the strain C. pylori (85P) is prepared so as to generate DNA fragments of high molecular weight; approximately 300µg of this chromosome DNA are submitted to a controlled partial digestion by the restriction endonuclease Sau3A, and deposited on sucrose gradient in such a way as to isolate selectively fragments of digestion comprised between 35 kb and 45 kb. 1.5µg of these fragments is ligated in vitro to the shuttle cosmid vector pILL575 linearized by the endonuclease BamHI, and dephosphorylated. pILL575 was constructed from the shuttle cosmid pILL550, described by Labigne-Roussel et al in J. Bacteriol. 169:5320-5323 (1987), into which was inserted the "cos" site of the lambda phage (fragment Bgl II of 1.7 kb of pERG153 inserted at site Pvu II of pILL550); like this cosmid, it may thus be mobilized by conjugation, code for a resistance to kanamycin which is expressed in both E. coli and Campylobacter (Km^r) and is replicated in both E. coli and C. jejuni due to the presence of two replication origins, one of which is functional in E. coli only, and the other solely in Campylobacter. The products of

this ligation are packaged in vitro in the lambda particles and then introduced by transfection in a strain of E. coli lodging a plasmid IncP capable of complementing in trans the transfer functions of the recombinant cosmids. The infected and kanamycin-resistant colonies of E. coli are immediately preserved individually at -80°C ; a cosmid genomic bank representing the entire genome of Campylobacter pylori is performed in E. coli by preserving approximately 500 independent clones.

Each of these recombinant cosmids is then transferred by conjugation of E. coli to a receptor strain of Campylobacter jejuni (C31), naturally urease.

Each of these kanamycin-resistant transconjugants is checked to see if synthesis of a urease by Campylobacter jejuni occurs.

One cosmid is isolated out of 106 carrying genetic information which gives Campylobacter jejuni C31 the ability to produce a urease. The size of the recombinant cosmid (IID2) is 54 kb. Figure 1 represents the relative position of the sites cleaved by the restriction endonucleases PstI, BamHI, SmaI and EcoRI on this sequence.

2) Identification of the gene(s) required for the production of urease in Campylobacter pylori.

Subcloning of the urease genes is carried out by performing, from the cosmid DNA IID2 prepared in E.

coli, partial digestion by endonuclease Sau3A so as to generate partial digestion fragments ranging from 8 kb to 15 kb. The purpose is to seek the smallest hybrid cosmid cloned in E. coli which confers the ability to produce a urease after its transfer by conjugation to receptor Campylobacter.

Out of the 37 tested, 4 plasmids carry the information required for the expression of the urease in Campylobacter jejuni: pILL586, pILL587, pILL589 and pILL590 (cf. Figure 2).

A comparison of the restriction maps of these four hybrids reveals the presence of a sequence of nucleotides of 4.2 kb common to the four hybrids, suggesting that this is the region required for the expression of the urease. A number of deletions, confirming these conclusions, were performed (cf. Figure 2).

Fragment EcoRI-Pst I of pILL590 was used as a probe in hybridization with the total DNA of C. pylori 85P so as to ensure that the different restriction fragments generated with BamHI and HindIII from pILL590 were present in 85P. The results obtained show that the sequence of 8 kb present in pILL590 was not rearranged in the course of the different cloning steps.

3) Sequence of nucleotides of the region associated with the expression of the urease.

The sequence of fragment HindIII lying between Sites H2 and H3 corresponds to the sequence of 294 nucleotides of the chain (I).

5 The ensuing sequence of 610 nucleotides of the chain (II) is internal to the fragment defined by sites H4 and B1 (Figure 2).

4) Specificity of the nucleotidic probes for the
10 detection of Campylobacter pylori.

Fragment EcoRI-PstI of 8 kb originating from pILL590 was used as radioactive probe in hybridization experiments.

32 Campylobacter pylori were tested in
15 hybridization on colonies and gave a positive signal; out of the 35 tested, none of the campylobacter jejuni, fetus or coli gave positive signals in standard and stringent hybridization conditions (50% formamide, 37°, 3XSSC).

20 In the same conditions, the 8 kb probe of C. pylori did not give a positive signal with the following micro-organisms, all of which are natural producers of urease:

25 Klebsiella oxytoca (3 strains), Klebsiella Pneumoniae (3 strains), Proteus mirabilis (3 strains), Proteus morganii (3 strains), Proteus rettgeri (3 strains), Proteus vulgaris (2 strains), Providencia stuartii (3 strains), Pseudomonas picketti (3 strains),

Yersinia enterocolitica (3 strains), 5 strains of Acinetobacter urease ⁺, 2 strains of Escherichia coli urease ⁺ and 3 strains of Citrobacter freundii urease ⁺.

5 The absence of false positive reaction with the fragment of 8 kb guarantees the specificity of the proposed nucleotidic probes.

A process of antibody preparation comprises:

- 10 - the immunization of an animal by means of a polypeptide as defined above, and
- the recovery of the antibodies formed, using standard techniques.

15 An appropriate chemical method for preparing the nucleic acids of the invention (these acids containing a maximum of 200 nucleotides - or pb in the case of bicatenary nucleic acids) comprises the following steps:

- 20 - synthesis of the DNA, using the B-cyanethyl phosphoramidite automated method described in Bioorganic Chemistry 4; 274-325, 1986,
- cloning of the DNAs thus obtained in an appropriate plasmid vector, and recovery of the DNAs by
25 hybridization with an appropriate probe.

A chemical method for the preparation of nucleic acids of a length in excess of 200 nucleotides - or pb

(in the case of bicatenary nucleic acids) comprises the following steps:

- 5 - assembly of chemically synthesized oligonucleotides equipped with different restriction sites at their ends, whose sequences are compatible with the amino acid chain of the natural polypeptide, according to the principle described in Proc. Nat. Acad. Sci. USA 80; 7461-7465, 1983,
- 10 - cloning of the DNAs thus obtained in an appropriate plasmid vector and recovery of the desired nucleic acid by hybridization with an appropriate probe.



CLAIMS

1. Sequence of nucleotides characterized in that it comprises in its structure a coding sequence for a protein with urease activity such as expressed by C.pylori, or in that it is capable of being hybridized
5 with the said coding sequence, which is enclosed in a fragment EcoR1 (ClaI, BamHI) - PstI (HindIII) of approximately 8 kb and whose enzymatic restriction map is represented in Figure 2.

2. Sequence of nucleotides according to claim 1,
10 characterized in that it is included in a part of a fragment of approximately 4.2 kb ($\pm 5\%$) which is marked by the limiting nucleotides situated respectively at a distance of approximately 0.55 kb upstream of site H2 and approximately 0.7 kb downstream of site B1, as
15 represented in Figure 2.

3. Recombinant nucleotides sequence, characterized in that it comprises a sequence according to one or other of claims 1 or 2, associated with a promoter which is capable of controlling the
20 transcription of the sequence, and a DNA coding sequence for the termination signals of the transcription.



4. Sequence of nucleotides according to one of claims 1 to 3, characterized in that it is capable of being hybridized with a nucleotidic probe formed from the sequence having:

5 - the following chain (I) of nucleotides:

10
AAGCTTTTAGGGGTGTTAGGGGTTTATCAAAAATCTAAAAAGGCGCTTCTCTCAAGCA
ATTGTGCTACAAACATGAGCAATTTAGCGCTTAAAGAATACTTAAAATOOCAAGATTTA
GAATTGAAGCATTTGCGGATTTGGGATAAGTTTGTGAGGGAATGCATGCGATTGAACAA
GCGAATTTTGGAGGCGAGCAAGCGGGCATATCATTTTLAGGATTAGCTTAAAACGGC
GATGGCTTTGGTGTGCGCTTTGCAAGTGAGCGGTTAGTGTAGAAAGTAAGCTT

15 - or the following chain (II bis) of nucleotides:

20
OCTAAAAGGATGACAACTATGTAAAAACAATTAAGGAGTAAGAATGAAAAGATTAGC
AGAAAAGAAATATGTTTCTATGTATGGTCTACTACAGGCGTAAAGTGAGATTGGGCGAT
CAGACTTGATGCTGAAGTGAACATGACTACACCATTTATGGGGAAGAGCTTAAATTGG
GTGGGGGTAAAACCGCTAAGAGAAGGCATGAGCGAATCTACAAACCTAGCAAAGAGAG
TTGGATTTAATTATCACTAAAGCTTTAATGTTGGATTACACCGGTATTATAAGCGGAT
ATTGGTATTAAAGATGGCAAAATGCGTGGCATTGGTAAAGGGGTAAACAAAGACATGCAA
GATGGGTTAAAACAATCTTAGGGTAGGTCTGCTACTGAAGCGTTAGCGGGTGAAGGT
TTGATOGTAAAGGCTGGTGGTATTGACACACATOCACCTTCATTTCAACCAACAAATC
25
OCTACAGCTTTTGAAGGGGTGTAACAACATGATTGGTGGTGAACCGGTCTGCTGAT
GGCACTAATGCGACTACTATCACTOCAGGCAGAAGAAATTTAAATGGATGCTCAGAGCG
GCTGAAGAATATTCTATGAATTTAGGTTCTTTGGCTAAAGGTAAAGCTTCTAAOGATGCG
AGCTTAGCGGATCAAATTGAAGCGGTGCGATTGGCTTTAAATTTCAOGAAGACTGGGGC
30
AOCCTCTCTGCAATCAATCATGGTT

- or the following chain (V) of nucleotides:



1
 ATGAAACTCAOOCCAAAAGGTTAGATAAGTTGATGCTOCTAOGCTGGAGAATTGGCT
 AAAAAAGCAAGAAAAAGGCATTAAAGCTTAACCTATGTAGAGCAGTAGCTTTGATTAGT
 5 GOOCATATTATCGAAGAAGCGAGAGCTGGTAAAAAGACTGCGGCTGAATTGATGCAAGAA
 GCGGCGACTCTTTTAAAAACAGATGATGTGATGGATGGGGTGGCAAGCATGATOCATGAA
 GTGGGTATTGAAGGATGTTTCTGATGGGACTAACTOGTAAOOGTGCAATAOOOCTATT
 GAGGOCATGGTAAATTAGTCTGCTGGTGAGTTGTCTCTTAAAAATGAAGACATCACTATC
 10 AAGCAAGGCAAAAAAGCGGTTAGGCGTGAAGTTAAAAATGTTGGGCAAGCAOOGGTTCAA
 ATCGGCTCACACTTTCATTCTTTGAAGTGAATAGATGCTAGACITTTGACAGAGAAAA
 ACTTTGGTAAAGCTTAGACATTGCGAGGGGACAGGGGTAGATTGTAGGCTGGGGAA
 15 GAAAAATOGTGAATTGATTGACATTGGGGGTAAACAGAGATCTTTGGATTTAAGCA
 TTGGTTGATAGCAAGCAGACAAGCAAGCAAAAAAATTGCTTTACACAGAGCTAAAGAG
 OGTGGTTTTCATGGGCTTAAAGGATGACAACTATGTAAACAATTAAGGAGTAA

20

- or the following chain (VII) of nucleotides:

25

30



1
 ATGAAAAGATTAGCAGAAAAGAAATATGTTTCTATGTATGGTCTACTACAGGOGATAA
 GTGAGATTGGGOGATAACAGACTTGATOGCTGAGTAGAATGACTACACCATTTATGGC
 GAAGAGCTTAAATTGGGTGGGGGTAAAACCTAAGAGAGGCTATGAGOCATCTAACAC
 5 OCTAGCAAAGAGAGTTGGATTTAATTATCCTAAOGCTTTAATOGTGGATTACAOOGGT
 ATTTATAAAGOGGATATTGGTATTAAGATGGCAAAATOGCTGGCATTTGGTAAAGGOGGT
 AACAAAGACATGCAAGATGGOGTTAAAAACAATCTTAGOGTAGGTCTGCTACTGAGOC
 TTAGODGGTGAGGTTTGATOGTAAOGGCTGGTGGTATTTGACACACATOCACCTCATT
 TCACOOCAACAATOOCTACAGCTTTTGCAAGOGGTGTACAAOCATGATTGGTGGTGG
 10 AOGGTCTGCTGATGGCACCTAATGOGACTACTATCCTOCAGGCAGAGAAATTTAAAA
 TGGATGCTCAGAGOGGCTGAGAAATATTCTATGAATTTAGGTTTCTTGGCTAAAGGTAC
 GCTTCTAAGATGOGAGCTTAGOOGATCAATTTGAAGOGGTGOGATTGGCTTTAAATTT
 CAGAGACTGGGGCAOACTOCTTCTGCAATCAATCATGOGTTAGATGTTGOGGCAAA
 TAGATGTGCAAGTGGCTATOCACACAGACACTTTGAATGAGOOGGTTGTGTAGAGAC
 15 ACTATGGCTGCTATTGCTGGAGGCACTATGCACACTTTTCACACTGAGGOGCTGGOSG
 GGCACGCTOCTGATATTATTAAAGTAGOOGGTGAACACACATTTCTTGOOGCTTCCACT
 AAOCOCACATOOCTTTCAOOGTGATACAGAGGAGAGACATGGACATGCTTATGGTG
 TGOCAOACTTGGATAAAAGCATTAAAGAAGATGTTCAGTGOGCTGATTCAAGGATOGC
 OCTCAAACATTGOGGCTGAGACACTTTGCATGACATGGGATTTTCTCAATCAOCAGT
 20 TCTGACTCTCAAGOGATGGGCOOGTGTGGGTGAAGTTATCCTAGAACTTGGCAAACAGCT
 GACAAAAACAAGAAAGAAATTTGGOOGCTTGAAAGAGAAAAGGOGATAAOGACAACCTTC
 AGGATCAAAGCTACTTGTCTAAATAACOCATTAAOOCAGOGATOGCTCATGGGATTAGC
 GAGTATGTAGGTTCAGTAGAGTGGGCAAGTGGCTGACTTGGTATTTGTGGAGTCCAGCA
 25 TTCTTTGGOGTGAAAOCAACATGATCATCAAAGGOGGATTCATTGOGTTAAGOCAAATG
 GOGATGOGAAOGCTTCTATOOCTAOCOCACAACOGGTTTATTACAGAGAAATGTTGGCT
 CATCATGGTAAAGCTAAATAOGATGCAACATCACTTTTGTGTCTCAAGOGGCTTATGAC
 AAAGGCATTAAAGAAGAATTAGGACTTGAAAGACAAGTGTGCOGGTAAAAAATTGCAGA
 AATATCCTAAAAAGACATGCAATTCAAOGACACTACTGCTCACATTGAAGTCAATOC
 30 GAACTTAOCATGTGTTGTTGGATGGCAAGAAGTAACTTCTAAAOAGOCATAAAGTG
 AGCTTGGOGCAACTCTTTAGCATTTTCTAG



- or the chains of nucleotides which are complementary respectively to the afore-mentioned chains (I) (II) (V) and (VII).

5. Sequence of nucleotides characterized in that
5 it comprises a chain of nucleotides according to claim 4, or in that it is constituted by at least part of this chain when it is able to recognize unequivocally the presence of a sequence according to claims 1 to 3.

6. Sequence of nucleotides corresponding,
10 according to the universal genetic code, to the following amino acids sequence (III):

**KLLGVLGVIYQSKNALSSQAVATNMSNLAKKEYLKSQDLKHCAGDKFVSEOMRLNK
ANFGGEQSGHIFSDYAKTGDGLVLCALQVSALVLESKL**

15

7. Sequence of nucleotides corresponding,
according to the universal genetic code, to the following amino acids sequence (IV):

20 **NMTTFMAKSLNSVAVKPLREGMSQSNPSKEELDIITNALIVDYTGTYKADIGIKDCK
LAGIGKGGNKMDGVKNLSVGPATEALAGEGLIVTAGGIDTHIHFI SPQOIPTAFRSG
VTIMIGGGTGPADGENATTTTPGRNKLWMLRAAEYSNIGFLAKGNASNDASLADQIE
AGAIGFKIHEDWGTTPSADHA**

25 8. Sequence of nucleotides corresponding,
according to the universal genetic code, to the following amino acids sequence (VI):

**MLLPKELDKMLHYAGELAKRKENGKLNVEVALISHIMEEARAGKCTAAELMOE
30 GRTLLKPDQVMDGVASMIHEVGDEAMFPDGIKLVVHTPIEANGKLVPGELFLKNEDITI
NEGKAVSVKVKNVGDRPVQIGSHEHFEVNRCLDEDREKTFGRDLIASGLAVRFEPEGE
EKSVELIDIGGNRRIFGENALVDROADNESKIALHRAKERGFHGAKSDDNYVKTKE**



9. Sequence of nucleotides corresponding, according to the universal genetic code, to the following amino acids sequence (VIII):

1
5 MKKSRKEYVSMYGPTTGDKVRLGDTDLAEVEHDYTYGEELKFGGGKTLREGM
SQSNPSKEELD LITNALVDYTGMYKADIGIKDGKIAGIGKGGNKDMQDGVKN
LSVGPATEALAGEGLVTAGGIDTHIHFI SPQQIPTAFASGVTTMIGGGTGPAD
GTNATTTTPGRRNLKWM LRAAEEYSMNLGLAKGNASNDASLADQIEAGAIGF
10 KIHEDWGTTPSAINHALDVADKYDVQVAIHDTLNEAGCVEDTMAAIAGRTHH
TFHTEGAGGGHAPDIIKVAGEHNILPASTNPTIPFTYNTEAEHMDMLMVCHLD
KSIKEDVQFADSRIRPQTIAAEDTLHDMGIFSTSSDSQAMGRVGEVITRTWQT
15 ADKNKKEFGRLKEEKGDNDNFRIKRYLSKYTINPAIAHGISEYVGSVEVGKVADL
VLWSPAFFGVKPNMIKGGFIALSOMGDANASIPTPQPVYYREMFAHHGKAKYD
ANITFVSQAAYDKGIKEELGLERQVLPVKNCRNITKKDMQFNDTTAHIEVNPETY
HVFVDGKEVT SKPANKVSLAQLFSIF_

569

20

10. Sequence of nucleotides, according to any one of claims 1 to 9, characterized in that it carries the information required for the production of a protein with urease activity, or of fragments of this protein which are capable of forming an immunological complex with antibodies respectively against the protein with urease activity as expressed by *C.pylori*, or against fragments of this protein.

11. Recombinant vector, notably recombinant
30 plasmid or cosmid, capable of transforming an appropriate host cell, the said vector containing a sequence of nucleotides according to any one of claims 1 to 10, possibly under the control of regulatory



elements allowing the expression of the fragment in the host cell.

12. Plasmid pILL590 carried by strain E.coli S17-1 registered at the CNCM on 16th August 1988 under
5 number I-795.

13. Micro-organism strain transformed by means of at least one vector according to claims 11 or 12 or by means of a sequence of nucleotides according to any one of claims 1 to 10.

10 14. Protein with urease activity, of the type expressed in C.pylori and its peptide fragments, corresponding, according to the universal genetic code, to the sequences of nucleotides according to any one of claims 1 to 10.

15 15. Protein with urease activity, of the type expressed in C.pylori, such as obtained through the transformation of host cells by means of a recombinant vector according to claims 11 or 12, culturing, in an appropriate environment, of the transformed host cells,
20 and recovery of urease from these cells or directly from the culture medium.

16. Polyclonal or monoclonal antibody, characterized in that it is directed against all or part of the protein or its fragments, according to
25 claims 14 or 15.

17. Polyclonal or monoclonal antibody, characterized in that it is directed against all or part of the sequence determined by the amino acids situated at positions 206 and 338 of sequence VIII,
30 according to claim 9.

18. Detection probe characterized in that it comprises a sequence of nucleotides according to any one of claims 1 to 10.



19. Process for in vitro screening of the possible presence of C.pylori in a sample likely to contain the said C.pylori, characterized in that it comprises the following steps:

- 5 - possibly, the preliminary amplification of a sequence according to any one of claims 1 to 12, likely to be contained in the sample, by means of primers likely to be linked respectively, on the one hand, to 5' end of a strand of the said sequence of nucleotides and, on the
10 other hand, to 3' end of the other strand of the said sequence of nucleotides,
- the coming into contact of the afore-mentioned biological sample with a probe according to claim 18, in conditions allowing the production of a
15 hybridization complex formed between the said probe and the said sequence of nucleotides,
- the detection of the afore-mentioned hybridization complex.

20. Process for in vitro screening of the possible presence of C.pylori in a sample likely to contain the said C.pylori, characterized in that it comprises:

- 25 - the coming into contact of the sample with an antibody according to claims 16 or 17, in conditions allowing the production of an immunological complex formed between the protein with urease activity produced by C.pylori and this antibody, and
- the detection of the immunological complex.

30 21. Necessary material or kit for the implementation of a method for in vitro screening of the possible presence of C.pylori in a sample, characterized in that it comprises:



- a given quantity of a nucleotidic probe according to claim 18,

- advantageously, an appropriate medium for the formation of a hybridization reaction between the sequence to be detected and the afore-mentioned probe,
- advantageously, reagents allowing the detection of the hybridization complexes formed between the sequence of nucleotides and the probe during the hybridization reaction.

22. Necessary material or kit for the implementation of a method for in vitro screening of the possible presence of C.pylori in a sample, characterized in that it comprises:

- a given quantity of an antibody according to claims 16 or 17,
- advantageously, an appropriate environment for the formation of an immunological reaction between at least a part of a protein with urease activity produced by a strain of C.pylori, and the antibody.
- advantageously, reagents allowing the detection of the immunological complexes formed between at least a part of the urease protein and the antibody during the immunological reaction.

23. Use as primers in amplification techniques of DNA, such as PCR or other types, of two sequences of nucleotides of approximately at least 20 nucleotides, included in one of the sequences according to any one of claims 1 to 10 and approximately 200 to 250 nucleotides apart, one of the said two sequences being capable of attaching itself to 5' end of a strand of the sequence to be amplified, and the other sequence being capable of attaching itself to 3' end of the other strand.



24. Pharmaceutical composition characterized in that it comprises antibodies according to claims 16 or 17 in association with a pharmaceutically acceptable vehicle.

5 25. Immunogenic composition characterized in that it comprises all or part of a protein according to claims 14 or 15 in association with a pharmaceutically acceptable vehicle.

10 26. Composition according to claim 25 characterized in that it comprises all or part of the sequence marked by the amino acids situated in positions 206 and 338 of sequence VIII according to claim 9.