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(54) Titre : UNE LIPOPROTEINE A SURFACE EXPOSEE DE CAMPYLOBACTER JEJUNI PARTICIPANT A
L'ADHESION AUX CELLULES HEP-2

(54) Title: A SURFACE-EXPOSED LIPOPROTEIN TO CAMPYLOBACTER JEJUNI INVOLVED IN ADHERENCE TO
HEP-2 CELLS

(57) **Abrégé/Abstract:**

The invention describes a novel protein of Campylobacter jejuni involved in the adhesion and invasion of the said bacterium, and more particularly novel nucleic acid molecules, novel proteins or molecules and application for which the nucleic acid molecules, proteins and substances are suitable. The product of this invention may be assembled into a kit for detecting the said bacterium by assaying for a J1pA protein or molecules, the presence of nucleic acid molecules or the presence of a lipoprotein or a lipid moiety on a polypeptide in a sample and directions for use. Composition for treatment of infectious diseases and vaccine are also described.



ABSTRACT OF THE DISCLOSURE

- 5 The invention describes a novel protein of *Campylobacter jejuni* involved in the adhesion and invasion of the said bacterium, and more particularly novel nucleic acid molecules, novel proteins or molecules and application for which the nucleic acid molecules, proteins and substances are suitable. The product of this invention may be assembled into a kit for detecting the said bacterium by
- 10 assaying for a JlpA protein or molecules, the presence of nucleic acid molecules or the presence of a lipoprotein or a lipid moiety on a polypeptide in a sample and directions for use. Composition for treatment of infectious diseases and vaccine are also described.

**Title: A SURFACE-EXPOSED LIPOPROTEIN TO *Campylobacter jejuni*
INVOLVED IN ADHERENCE TO Hep-2 CELLS**

5 FIELD OF THE INVENTION

The invention relates to a novel surface-exposed lipoprotein, JlpA, specific to *Campylobacter jejuni*.

BACKGROUND OF THE INVENTION

Campylobacter jejuni is a Gram-negative, spiral,
10 microaerophilic bacterium that exists as a commensal organism in the intestinal tracts of a variety of wild and domestic animals. It is a leading cause of acute bacterial enterocolitis in humans worldwide (Altekruse, 1999). Clinical symptoms of campylobacteriosis range from a mild watery diarrhea to bloody diarrhea with fever, abdominal cramps, and presence of fecal leukocytes. C.
15 jejuni infection in human is often associated with undercooked chicken and turkey, red meat, raw milk, and untreated water. C. jejuni has also been strongly implicated in the etiopathogenesis of Guillain-Barre Syndrome (GBS), a neurological disease that results in respiratory compromise and death. Pathogenic mechanisms of infection caused by C. jejuni are still not well
20 understood, but are known to involve colonization, adhesion, and likely also cellular invasion and toxin production in the human intestinal tract (Ketley, 1997).

Adherence of bacteria to host cells is a critical step in an infection, which is generally recognized as a multifactoral process and mediated
25 by adhesins on the bacterial surface. Bacterial adhesins are either assembled into hair-like appendages called pili or directly associated with cell surface (Soto and Hultgren, 1999). Although pili have been demonstrated to be adhesins in many bacterial pathogens, such a structure has not been confirmed in C. jejuni. A most recent study showed that some environmental stimuli induce a pilus-like
30 appendage on the cell surface of C. jejuni and C. coli. However, this cell appendage does not play a role in adherence of C. jejuni to epithelial cells (Doig,

et al., 1996). Therefore, the adhesins of *C. jejuni* might be directly associated with the cell surface. Fauchere et al. (1989) reported that several outer membrane proteins of *C. jejuni*, including the major cell-binding factor CBF1, specifically bind to HeLa cells. Later studies showed that CBF1 is identical to PEB1, one of the PEB antigenic proteins (Kervella, et al., 1993). Genetic analysis indicated that PEB1 is homologous to a component of the ABC transport system in Gram-negative bacteria (Pei and Blaser, 1993). The ability of adherence of *C. jejuni* to epithelial cells was reduced 50- to 100-fold but not totally abolished when the *peb1* gene was disrupted, suggesting the possible presence of other adhesins in *C. jejuni*. Konkak et al. (1997) identified a 37 kDa outer membrane protein from *C. jejuni*, named CadF, which specifically binds to fibronectin, a component of extracellular matrix of mammalian cell. The fibronectin binding activity of cadF mutants was reduced 3- to 6-fold. DE Melo and Pechère (1990) discovered four specifically binding proteins with apparent molecular mass of 28, 32, 36, and 42 kDa when they used the cell surface extracts of *C. jejuni* to bind HEp-2 cells. PEB1 (28 kDa) and CadF (37 kDa) possibly correspond to two of these four proteins. In addition, a 59 kDa outer membrane protein and the 43 kDa major outer membrane protein (MOMP) were reported to bind to the membrane of epithelial cells (Schroder and Moser, 1997; Moser et al., 1997).

20 **SUMMARY OF THE INVENTION**

The present inventors have isolated a novel cell surface adhesin of *C. jejuni* which has homology to a rhoptry protein of the malaria parasite.

The novel cell surface adhesin, JlpA, plays an important role in the adhesion of *C. jejuni* to Hep-2 cells. This polypeptide is released into the culture medium during the growth and binds Hep-2 cells. JlpA is the first protein presently reported in *Campylobacter* that shares homology with eukaryotic protein. The glycine-acid extraction and proteinase K digestion experiments indicate that JlpA is a surface-exposed protein in the said bacterium.

Accordingly, the present invention provides a purified and isolated polypeptide comprising a sequence of 1116-bp of the open reading frame.

The invention also contemplates a jlpA gene encoding a polypeptide, JlpA, of 372 amino acid residues with a molecular mass of 42.3 kDa. JlpA contains a typical signal peptide and a lipoprotein processing site at the N-terminus. JlpA is a surface exposed lipoprotein. JlpA is loosely associated with the cell surface. JlpA binds specifically to Hep-2 cells.

The invention also contemplates truncations of the protein and analogues, homologs and isoforms of the protein and truncations thereof.

The protein may be conjugated to produce other proteins such as fusion proteins. Such proteins can be generated, for example, by the synthesis of N-terminal and C-terminal fusion proteins.

The invention further contemplates the creation of antibodies to the epitope of the polypeptide of the invention, preferably the creation of polyclonal antibodies. Furthermore, it is contemplated that such antibodies can be used to detect the presence of JlpA protein and like polypeptides under a variety of conditions and environments.

A kit for the detection of JlpA epitope preferably a monoclonal antibody and its use is also provided. The kit may also contain reagents which are needed to bind the antibody to the polypeptide protein in the sample.

The nucleic acid sequence for JlpA of the invention allows those skilled in the art to construct nucleotide probes for the use and detection of same, related or analogous polypeptides in a variety of samples including food, the environment and other biological materials.

The invention further provides for a kit for detection of the presence of nucleic acid molecules having a sequence encoding a polypeptide for JlpA, a related or analogous polypeptide. The kit comprises a nucleotide probe which hybridizes with the nucleic acid molecule, reagents required for

hybridization of the nucleotide probe with the nucleic acid molecule and directions for its use.

The nucleic acid sequence can be used in the polymerase chain reaction [PCR] to amplify the nucleic acid molecule of this invention.

5 Accordingly, the invention relates to a method of determining the presence of a nucleic acid molecule having a sequence encoded a JlpA protein or a predetermined part of JlpA protein in a sample.

The invention further relates to a kit for the determining the presence of a nucleic acid molecule bearing the sequence of JlpA protein or a
10 predetermined part of the polypeptide in a sample. The kit comprises of primers which are capable of amplifying the nucleic acid molecule in a PCR reaction, reagents required to amplify the nucleic acid molecule thereof in an amplification reaction, preferably in a PCR reaction, means for assaying the amplified sequence and directions for the use of the kit.

15 The nucleic acid molecules of the invention may also be used to assay for a substance which inhibit adherence or invasion of *C. jejuni*. As such, the invention provides for a method for assaying for a substance that interferes with a JlpA polypeptide. The method may be used, for example, to assay for a substance that impacts on the growth or pathogenicity of *C. jejuni*.

20 The substances identified using the method of invention, antibodies and antisera molecules may be used to inhibit the binding, adhesion or invasion of *C. jejuni*. Accordingly, the substances may be used in the treatment of infectious diseases caused by *C. jejuni*. There is in this respect provided a method of inhibiting the binding of *C. jejuni* comprising
25 administering to an animal an effective amount of a substance capable of inhibiting the adherence of a JlpA protein in an animal in need thereof. As such, these substances may be formulated into pharmaceutical composition for the administration to subjects.

30 Other objects, features and advantages of the present invention will become apparent from the following detailed description. It

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should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this
5 detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 (SEQ. ID. NO.: 1) shows the sequence of 5' nucleotide
10 sequence and the N-terminus deduced amino acid sequence of the jlpA;

Figure 2 (SEQ. ID. NOS.: 10 and 11) shows the alignment shown is modified from the alignment obtained from a BlastP database search;

Figure 3 shows the expression of JlpA in *E. Coli*;

Figure 4 shows surface localization of JlpA in *C. jejuni* TGH9011;

15 Figure 5 shows release of JlpA during growth;

Figure 6 shows genetic organization of hipO-jlpA region and construction of jlpA mutants;

Figure 7 shows specific binding of purified JlpA to Hep-2 cells; and

20 Figure 8 shows the presence of jlpA in *Campylobacter* species.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The following standard abbreviations for the amino acid residues are used throughout the specifications: A, Ala - alanine; C, Cys - cysteine; D, Asp- aspartic acid; E, Glu - glutamic acid; F, Phe - phenylalanine; G, Gly - glycine;
25 H, His - histine; I, Ile - isoleucine; K, Lys - lysine; L, Leu - leucine; M, Met - methionine; N, Asn - asparagine; P, Pro - proline; Q, Gln - glutamine; R, Arg - arginine; S, Ser - serine; T, Thr - threonine; v, Val - valine; W, Trp - tryptophan; Y, Try - tyrosine; and p.Y., P.Tyr - phosphotyrosine.

Administration of an "effective amount" of the compounds of
30 the present invention is defined as an amount effective, at dosages and for

periods of time necessary to achieve the desired result. The effective amount of a compound of the invention may vary according to factors such as the disease state, age, sex, and weight of the animal. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses
5 may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The term "animal" as used herein includes all members of the animal kingdom, including humans. Preferably, the animal to be treated is a human.

10 The description of the invention is divided into three sections. They are the following sections: [1] novel nucleic acid molecule; [2] novel proteins[s]; and [3] application for which the nucleic acid molecules, proteins and substances identified herein are suitable.

[1] Nucleic acid molecules of this invention:

15 The present invention relates to an isolated nucleic acid of *C. jejuni* encoding a polypeptide associated with adhesion. The term "isolated" refers to nucleic acid substantially free of cellular material or culture material when produced by recombinant DNA technology, or chemical precursors, or other chemicals when chemically synthesized. The term "nucleic acid" is
20 intended to include DNA and RNA and can be either double stranded or single stranded.

In the embodiment of the invention, an isolated nucleic acid molecule is provided having a sequence which encodes a protein having an amino acid as shown in Figure 1.

25 Preferably, the purified and isolated nucleic acid molecule comprises

[a] a nucleic acid sequence as shown in Figure 1, where T can also be U;

[b] nucleic acid sequence complementary to [a];

30 [c] nucleic acid sequences which are homologous to [a] and [b];

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[c] a fragment of [a] to [c] that is at least 15 bases, preferably 20 to 30 bases, and which will hybridize to [a] and [c] under stringent hybridization conditions; or

[d] a nucleic acid molecule differing from any of the nucleic acids of [a] to [c] in codon sequences due to the degeneracy of the genetic code.

The invention includes nucleic acid molecules comprising nucleic acid sequences having substantially sequence homology with the nucleic acid sequence as shown in Figure 1. The term "sequences having substantial sequence homology" means those nucleic acid sequences which have slight or inconsequential sequence variations from these sequences, i.e. the sequences function in substantially the same manner to produce functionally equivalent proteins. The variations may be attributed to local mutations or structural modifications.

Another aspect of the invention provides a nucleic acid molecule, and fragment thereof having at least 15 bases, which hybridizes to the nucleic acid molecules of the invention under hybridization conditions, preferably stringent hybridization conditions. A person skilled in the art is knowledgeable about appropriate stringency conditions which promote DNA hybridizations and the techniques available thereof.

Isolated and purified nucleic acid molecules having sequences which differ from the nucleic acid sequences of JlpA due to degeneracy in the genetic code are also within the scope of this invention.

The determination of whether a particular nucleic acid molecule encodes a novel protein of the invention may be accomplished by expressing the cDNA in an appropriate host cell by standard techniques, and testing the activity of the protein using the methods as described herein. In addition, regulatory elements may be identified in the DNA through use of constructs that may identify proteins interacting with the elements by use of techniques known in the art.

The sequence of a nucleic acid molecule of the invention may be inverted relative to its normal presentation for transcription to produce an

antisense nucleic acid molecule. Preferably, an antisense sequence is constructed by inverting a region preceding the initiation codon or unconverted region. In particular, the nucleic acid sequence contained in the nucleic acid molecule of the invention or a fragment thereof, preferably a nucleic acid sequence shown in
5 Figure 1 may be inverted relative to its normal presentation for transcription to produce antisense nucleic acid molecules.

The antisense nucleic acid molecule of the invention or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the
10 biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene, i.e. phosphorothioate derivatives and acridine substituted nucleotides. The antisense sequences may be produced biologically using an expression vector induced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which
15 antisense sequences are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the vector is introduced.

The invention also provides nucleic acids encoding fusion proteins comprising a novel protein of the invention and a selection protein, or
20 a selectable marker protein [see below].

[2] Novel proteins of invention

The invention broadly contemplates an isolated protein characterized in that it has part or all of the primary structural conformation [i.e. continuous sequence of amino acid residues] of a novel protein encoded by the
25 jlpA gene of the invention. In the embodiment of the invention, an isolated protein is provided which has the amino acid sequence as shown in Figure 1.

Within the context of the present invention, a protein of the invention may include various structural forms of the primary protein which retain biological activity. For example, a protein of the invention may be in the
30 form of acid or basic salts or in neutral form. In addition, individual amino acid residues may be modified by oxidation or reduction.

In addition to the full length amino acid sequence the protein of the present polypeptide of the invention may also include truncation of the polypeptide, analogues and homologs of the polypeptide. Truncations may comprise peptides of at least fifteen amino acid residues.

5 Analogues of the proteins having the amino acid sequence shown in Figure 1 and / or truncations thereof as described herein, may include, but not be limited to an amino acid sequence containing one or more amino acid substitutions, insertions and / or deletions. Amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions
10 involve replacing one or more amino acids of the proteins of the invention with amino acids of similar charge, size and / or hydrophobic characteristics. When only conserved substitutions are made the resulting analogue should be functionally equivalent. Non-conserved substitutions involve replacing one or more amino acids of the amino acid sequence with one or more amino acids
15 which possess dissimilar charge, size and / or hydrophobicity characteristics.

One or more amino acid insertions may be introduced into the amino acid sequences shown in Figure 1. Amino acid insertions may consist of single amino acid residues or sequential amino acids ranging from 2 to 15 amino acids in length. For example, amino acid insertions may be used to
20 destroy target sequences so that the protein is no longer active. This procedure may be used *in vivo* to inhibit the activity of a protein of the invention.

Deletions may consist of the removal of one or more amino acids, or discrete portions from the amino acid sequence shown in Figure 1. The deleted amino acids may or may not be contiguous. The lower limit length of
25 the resulting analog with deletion mutation is about 10 amino acids.

Analogues of a protein of the invention may be prepared by introducing mutations in the nucleotide sequence encoding the protein. Mutations in nucleotide sequences constructed for expression of analogue of a protein of the invention must prevent the reading frame of the coding
30 sequence. Furthermore, the mutations will preferably not create complementary regions that could hybridize to produce secondary mRNA structures, such as

loops or hairpins, which could adversely affect translation of the receptor mRNA.

Mutations may be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites
5 enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstruction sequence encodes an analogue having the desired amino acid insertion, substitution or deletion.

Alternatively, oligonucleotide-directed site specific mutagenesis procedures may be employed to provide an altered gene having
10 particular codons altered according to the substitution, deletion or insertion required. Deletion or truncation of a protein of the invention may also be constructed by utilizing convenient restriction endonuclease sites adjacent to the desired deletion. Subsequent to restriction, overhangs may be filled in, and the DNA relegated. Methods of making the alterations are set forth including by
15 Sambrook et al. [11].

The proteins of the invention also include homologs of the amino acid sequence shown in Figure 1. Such homologs are proteins whose amino acid sequences are comprised of amino acid sequences that hybridize under stringent hybridization conditions with a probe used to obtain a protein
20 on the invention. Homologs of a protein of the invention will have the same regions which are characteristic of the protein.

In heterologous species of *C. jejuni*, a homologous protein includes a protein with an amino acid sequence having at least 30%, preferably 40-50% identity with the amino acid sequence as shown in Figure 1. In
25 homologous species of *C. jejuni*, a homologous protein includes a protein with an amino acid sequence having at least 70% preferably 80-90% identity with the amino acid sequence as shown in Figure 1.

The invention also contemplates isoforms of the proteins of the invention. An isoform contains the same number and kinds of amino acids
30 as a protein of the invention, but the isoform has a different molecular

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structure. The isoforms contemplated by the present invention are those having the same properties as a protein of the invention as described herein.

The present invention also includes a protein of the invention conjugated with a selected protein, or a selection marker protein to
5 produce fusion proteins. Additionally, immunogenic portions of the protein of the invention are within the scope of the invention.

The protein of the invention [including truncation, analogues etc.] may be prepared using recombinant DNA methods. Accordingly, the nucleic acid molecules of the present invention having a sequence which
10 encodes a protein of the invention may be incorporated in a known manner into an appropriate expression vector which ensures good expression of the protein. Possible expression vectors include but are not limited to cosmids, plasmids or modified viruses [e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses], so long as the vector is comparable
15 with the host cell used. The expression vectors are "suitable for transformation of a host", means that the expression vectors contain a nucleic acid molecule of the invention and regulatory sequences selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid molecules. Operatively linked is intended to mean that the nucleic acid is linked to
20 regulatory sequences in a manner which allows expression of the nucleic acid.

The invention therefore contemplates a recombinant expression vector of the invention containing a nucleic acid molecule of the invention, or a fragment thereof, and the necessary regulatory sequences for the transcription and translation of the inserted protein-sequence. Suitable
25 regulatory sequences may be derived from a variety of sources, including bacterial, fungal or viral genes [12]. Selection of the appropriate regulatory sequences is dependent on the host cell chosen and may be readily accomplished by one skilled in the art. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication,
30 additional DNA restriction site, enhancers and sequences conferring inducibility of transcription may be incorporated into the expression vector. It will also be

appropriate that the necessary regulatory sequences may be supplied by the native protein and / or its flanking regions.

The invention further provides a recombinant expression vector comprising a DNA nucleic acid molecule of the invention cloned into
5 the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequencer in a manner which allows for expression, by transcription of the DNA molecule, of an RNA molecule which is antisense to a nucleotide sequence comprising the nucleotides as shown in Figure 1. Regulatory sequences operatively linked to the antisense nucleic acid
10 can be chosen which direct the continuous expression of the antisense RNA molecule.

The recombinant expression vector of the invention may also contain a selection marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention.
15 Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs, β -galactosidase, chloramphenicol acetyltransferase or firefly luciferase. The procedures are known to one skilled in the art. It is appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

20 The recombinant expression vector may also contain genes which encode a fusion moiety which provides increased expression of the recombinant protein; increased solubility of the recombinant protein; and aid in the purification of a target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target
25 recombinant protein to allow separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein.

Recombinant expression vectors can be introduced into the host cells to produce a transformant host cell. The term "transformant host cell" is intended to include prokaryotic and eukaryotic cells which have been
30 transformed or transfected with a recombinant expression vector of the invention. The terms "transformed with", "transfected with", "transformation"

and "transfection" are intended to encompass introduction of nucleic acid [e.g. a vector] into a cell by one of many possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can
5 be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofectin, electroporation or microinjection. Appropriated methods for transforming and transfecting host cells can be found in a variety of matters including in Sambrook et al.[11].

10 Suitable host cells including a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins of the invention may be expressed in bacterial cells such as *E. coli*, insect cells [using baclovirus], yeast cells or mammalian cells. Other suitable hosts can be found in other matters including in Goeddel [12].

15 The proteins of the invention may also be prepared by chemical synthesis using techniques well known to a person skilled in the art of chemistry.

[3] Application for which the nucleic acid molecules, proteins and substances identified herein are suitable

20 **[a] detection of nucleic acid molecules, antibodies and diagnostic application**

 The nucleic acid molecules of the invention, allow those skilled in the art to construct nucleotide probes for use in the detection of nucleotide sequences in a sample. A nucleotide probe may be labelled with a detectable marker such as a radioactive label which provides for an adequate
25 signal and has sufficient half life such as ^{32}P , ^3H , ^{14}C or the like. Other detectable markers which may be used include antigens that are recognized by a specific labelled antibody, fluorescent compound, enzymes, antibodies specific for a labelled antigen and chemiluminescent compounds. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to
30 the nucleotide to be detected and the amount of nucleotide available for hybridization.

The nucleotide probe may be used to detect genes that encode proteins related to or analogous to JlpA proteins of the invention.

Accordingly, the present invention also relates to a method of detecting the presence of the nucleic acid molecule encoding a JlpA protein of the invention in a sample comprising contacting the sample under hybridization conditions with one or more of nucleotide probes which hybridize to the nucleic acid molecule and are labelled with a detectable marker, and determining the degree of hybridization between the nucleic acid molecule in the sample and the nucleotide probes.

10 In an embodiment of the invention, a method is provided for detecting *C. jejuni* in a sample comprising contacting the sample with a nucleic acid molecule containing a nucleic acid sequence encoding a JlpA protein, or a fragment thereof, under conditions which permit the nucleic acid molecule to hybridize with a complementary sequence in the sample to form a hybridization product, and assaying for hybridization product.

Hybridization conditions which may be used in the methods of the invention are known in the art and are described for example in Sambrook[11]. The hybridization product may be assayed using techniques known in the art. The nucleotide probe may be labelled with a detectable marker as described herein and the hybridization product may be assayed by detecting the detectable marker or the detectable change produced by the detectable marker.

The nucleic acid molecule of the invention also permits the identification and isolation, or synthesis of nucleotide sequences which may be used as primers to amplify a nucleic acid molecule of the invention, for example in PCR. The primers may be used to amplify the genomic DNA of other bacterial species. The PCR amplification sequence can be examined to determine the relationship between the various JlpA genes.

It will be appreciated that the primers may contain non-complementary sequences provided that a sufficient amount of the primer contains a sequence which is complementary to a nucleic acid molecule of the

invention or oligonucleotide fragment thereof, which is to be amplified. Restriction site linkers may also be incorporated into the primers allowing for digestion of the amplified products with the appropriate restriction enzymes facilitating cloning and sequencing of the amplified product.

5 In an embodiment of the invention a method of determining the presence of a nucleic acid molecule having a sequence encoding a protein of the invention is provided comprising treating the sample with primers which are capable of amplifying the nucleic acid molecule or predetermining oligonucleotide fragment thereof in a polymerase chain reaction to form
10 amplified sequences, under conditions which permit the transformation of amplified sequences and assaying for amplified sequences.

The amplified products can be isolated and distinguished based on their respective sizes using techniques known in the art. For example, after amplification, the DNA sample can be separated on an agarose gel and
15 visualized, after staining with ethidium bromide, under ultra violet [UV] light. DNA may be amplified to a desired level and a further extension reaction may be performed to incorporate nucleotide derivatives having detectable markers such as radioactive labelling or biotin labelled nucleoside triphosphates. The primers may also be labelled with detectable markers as discussed above. The
20 detectable markers may be analyzed by restriction and electrophoretic separation or other techniques known in the art.

The conditions which may be employed in the methods of the invention using PCR are those which permit hybridization and amplification reactions to proceed in the presence of DNA in a sample and appropriate
25 complementary hybridization primers. Conditions suitable for the polymerase chain reaction are known in the art. It is appreciated that other techniques may be used including Ligase Chain Reaction [LCR] and NASBA.

A JlpA protein of the invention can be used to prepare antibodies specific for the protein. Antibodies can be prepared which bind a
30 distinct epitope in an unconserved region of the protein. An unconserved region of the protein is one which does not have substantial sequence homology

to other proteins. Alternatively, a region from a well-characterized domain can be used to prepare an antibody to a conserved region of a protein of the invention. Antibodies having specificity for a protein of the invention may also be raised from fusion proteins.

5 Conventional methods can be used to prepare antibodies. For example, by using a peptide of a protein of the invention, polyclonal antisera or monoclonal antibodies can be made using standard methods known to one skilled in the art.

10 The term "antibody" as used herein is intended to include fragments thereof which also specifically react with a protein, of the invention or peptide thereof. Antibodies can be fragmented using conventional techniques and the fragments screened for utility by techniques known to one skilled in the art. For example, F[ab']₂ fragments can be generated by treating antibody with pepsin. The resulting F[ab']₂ fragment can be treated to reduce disulphide bridges
15 to produce Fab' fragments.

 Chimeric antibody derivatives, i.e. antibody molecules that combine a non-human animal variable region and a human constant region are also contemplated within the scope of the invention. Chimeric antibody molecules can include, for example, the antigen binding domain from an
20 antibody of a mouse, rat or other species, with human constant region. Conventional methods may be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes a JlpA protein [see for example [15]].

 Monoclonal or chimeric antibodies specifically reactive with a
25 protein of the invention as described herein can be further humanized by producing human constant region chimeras, in which parts of the variable regions, particularly the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. Such immunoglobulin molecules may be made by techniques
30 known in the art.

Specific antibodies or antibody fragments reactive against a protein of the invention may also be generated by screening expression libraries encoding immunoglobulin genes, or portion thereof, expressed in bacteria with peptides produced from the nucleic acid molecules of the present invention. For
5 example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries.

The antibodies may be labelled with a detectable marker including various enzymes, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish
10 peroxidase, biotin, alkaline phosphatase, β -galactoside or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material includes S-35,
15 Cu-64, Ga-67, Zr-89, Ru-97, Tc-99m, Rh-105, Pd-109, In-111, I-125, I-131, Re-186, Au-198, Au-199, Pb-203, At-211, Pb-212 and Bi-212. the antibodies may also be labelled or conjugated to one partner of a ligand binding pair. Representative examples include avidin-biotin and riboflavin-riboflavin binding protein. methods for conjugating or labelling the antibodies discussed above with the
20 representative labels set forth above may be readily accomplished using technique known to one skilled in the art.

The antibodies reactive against proteins of the invention [e.g. enzyme conjugates or labelled derivatives] may be used to detect a protein of the invention in various samples, for example they may be used in any known
25 immunoassays which rely on the binding interactions between any antigenic determinant of a protein of the invention and the antibodies. Examples of such assays are radioimmunoassays, enzyme immunoassays [e.g. ELISA], immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination and histochemical tests. Thus, the antibodies may be used to
30 identify or quantify the amount of a protein of the invention in a sample in order to diagnose *C. jejuni* infections.

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A sample may be tested for the presence or absence of a pathogenic *C. jejuni* serotype by contacting the sample with an antibody specific for an epitope of JlpA protein which antibody is capable of being detected after it becomes bound to a JlpA protein in the sample, and assaying for antibody bound
5 to a JlpA protein in the sample, or unreacted antibody.

In a method of the invention a predetermined amount of a sample or concentrated sample is mixed with antibody or labelled antibody. The amount of antibody used in the process is determined upon the labelling agent chosen. The resulting protein bound to antibody or labelled antibody may be
10 isolated by conventional isolation techniques, for example, salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, or combination thereof.

For example, one can use labelled and unlabelled or soluble or insoluble antibody in a given assay mix, e.g. the antibodies can be bound to
15 suitable carriers such as Sepharose or agarose beads; a labelled antibody can be assessed in a sample as it binds to the protein of the invention or one can assess the unreactive antibody; and unlabelled antibody can be determined by measuring the amount of antibody bound to *C. jejuni* serotype using substances that interact specifically with the antibody to cause agglutination or
20 precipitation.

The reagents suitable for applying the methods of the invention may be packaged into convenient kits providing the necessary materials, packaged into suitable containers. Such kits may include all the reagents required to detect a pathogenic *C. jejuni* serotype in a sample by means
25 of the methods described herein, and optionally suitable supports useful in performing the methods of the invention.

In one embodiment of the invention the kit contains a nucleotide probe which hybridizes with a nucleic acid molecule of the invention, reagents required for hybridization of the nucleotide probe with the
30 nucleic acid molecule, and directions for its use. In another embodiment of the invention the kit includes antibodies of the invention and reagents required for

binding of the antibody to a protein specific for a pathogenic *C. jejuni* serotype in a sample. In still another embodiment of the invention, the kit includes primers which are capable of amplifying a nucleic acid molecule of the invention or a predetermined oligonucleotide fragment thereof, all the reagent
5 required to produce the amplified nucleic acid molecule or predetermined fragment thereof in the polymerase chain reaction, and means for assaying the amplified sequences.

The methods and kits of the present invention have many uses. For example, the methods and the kits of the present invention may be
10 used to detect a pathogenic *C. jejuni* serotype in any medical or veterinary sample suspected of containing the said organism. Samples that may be tested included bodily materials such as blood, urine, serum, tears, saliva, feces, tissues and the like. In addition to human samples, samples may be taken from mammals such as non-human primates, etc. Further, water and food samples
15 and other environmental samples and industrial wastes may be tested.

Before the samples are tested using the invention described herein, the samples may be concentrated using the techniques known in the art, such as centrifugation and filtration. For the hybridization and / or PCR- based methods using the invention described herein, nucleic acids may be extracted
20 from cell extracts of the test sample using techniques known in the art.

Substances that affect adherence and / or invasion of *C. jejuni*

A JlpA protein of the invention may also be used to assay for substances which affect adherence and / or invasion of the said organism. Accordingly, the invention provides a method for assaying for a substance that
25 affects adherence and / or invasion of *C. jejuni* comprising mixing a protein of the invention with a test substance which is suspected of affecting the expression or activity of the protein, and determining the effect of the substance by comparing to a control.

The reagents suitable for applying the methods of the
30 invention to identify substances that affect adherence and / or invasion of *C. jejuni* may be packaged into convenient kits providing the necessary materials

packaged into suitable containers. The kits may also include suitable supports useful in performing the methods of the invention.

Pharmaceutical Compositions and Methods of Treatment

The substances identified by the methods described herein, antisense nucleic acid molecules, and antibodies, may be used for reducing adherence and / or invasion of *C. jejuni* and accordingly may be used in the treatment of infectious diseases caused by *C. Jejuni*.

The substances identified using the methods described herein and antibodies may be formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effect. The substances may be administered to living organisms including humans and animals. Administration of a therapeutically active amount of the pharmaceutical compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired results. For example, a therapeutically active amount of a substance may vary according to factors such as the disease state, age, sex and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regime may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active substance may be administered in a convenient manner such as by injection [subcutaneous, intravenous, etc.], oral administration, inhalation, transdermal application or rectal administration. Depending on the route of administration, the active substance may be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

The compositions described herein can be prepared by *per se* known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective

quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences [Remington's Pharmaceutical Sciences, Marck Publishing Company, Easton, Pa., USA 1985]. On this basis, the
5 compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

Recombinant molecules comprising an antisense sequence or
10 oligonucleotide fragment thereof, may be directly introduced into cells or tissues *in vivo* using delivery vehicles such as retroviral vectors, adenoviral vectors and DNA virus vectors. They may also be introduced into cells *in vivo* using physical techniques such as microinjection and electroporation or chemical methods such as coprecipitation and incorporation of DNA into liposomes.
15 Recombinant molecules may also be delivered in the form of an aerosol or by lavage.

The utility of the substances, antibodies, antisense nucleic acid molecules and compositions of the invention may be confirmed in animal experimental model systems.

20 Vaccines

The present invention relates to a vaccine against an infectious disease caused by *C. jejuni* comprising a carrier strain having an amount of a JlpA protein associated with its surface which is effective to provide protection against *C. jejuni*. "Infectious disease" refers to any disease or
25 condition due to the action of *C. jejuni*. The vaccines may be used for the prophylaxis or active immunization and treatment of infectious diseases caused by the said organism.

The carrier strain may be selected so that it is incapable of multiplying *in vivo*. Carrier strains are obtained through selection of variants
30 which occur naturally, or using conventional means known to those skilled in

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the art. Examples of suitable carrier strains are *Shigella* species, *Salmonella* species, *S. Typhimurium* species, *Vibrio* species and *Escherichia* species.

The invention also relates to a method of preparing a vaccine against an infectious disease caused by *C.jejuni* comprising associating with the
5 cell surface of a carrier strain a JlpA protein or portion thereof which is effective to provide protection against *C.jejuni*. A JlpA protein or portion thereof may be associated with the cell surface of a carrier strain using conventional methods.

The vaccine may be multivalent vaccine and additionally contain immunogens related to other infectious diseases in a prophylactically or
10 therapeutically effective manner. Multivalent vaccines against infectious diseases caused by different infectious agents may contain a carrier strain having amounts of antigens associated with their surfaces which are effective to provide protection against the infectious agents.

A multivalent vaccine may comprise at least two carrier
15 strains each having different immunogens associated with different infectious agents. A multivalent vaccine may contain a carrier strain having at least two different immunogens associated with different infectious agents. Thus, a carrier strain may contain immunogens relating to *C.jejuni* and other pathogenic microorganisms.

20 The vaccine of the invention contains an immunologically effective amount of the carrier strain[s] with the integrated JlpA protein. The optimum amounts of cells per dosage unit depends on the nature of the infection against which protection is required, the characteristics of the animals to be protected and other factors known to persons skilled in the art.

25 In addition to the carrier strain[s], the vaccine may comprise an immunologically acceptable carrier such as aqueous diluents, suspending aids, buffers, excipient and one or more adjuvants known in the art. The vaccine may also contain preservatives such as sodium azide, thimersol, beta propiolactone and binary ethyleneimine.

30 The vaccines of the invention can be intended for administration to animals, including mammals, avian species and fish;

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preferably humans and various other mammals, including bovines, equines and swine.

The vaccines of the invention may be administered in convenient manner, such as intravenously, intramuscularly, subcutaneously, 5 intraperitoneally, intranasally or orally. The dosage will be dependent on the nature of the infection, on the desired effect and on the chosen route of administration and other factors known to persons skilled in the art.

The JlpA proteins and portions thereof of the invention are also useful for preparing antibodies which may be used as a means of passive 10 immunization.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and 15 equivalent arrangements included within the spirit and scope of the appended claims.

EXAMPLES

General DNA manipulation and analysis

Plasmid and chromosomal DNA were isolated and 20 manipulated by using standard techniques (Ausubel, et al., 1989; Sambrook et al., 1989). Transformation of plasmids into E. coli strains was performed by the method of Inoue et al. (1990). PCR amplification was carried out using Campylobacter chromosomal DNA as a template for 26 cycles at 95°C, 20 sec; 50°C, 20 sec; and 72°C, 1 min. DNA was sequenced using Sequenase Version 25 2.0 (United States Biochemical Corp.) and [α -32P] dATP (ICN Biomedicals Inc.) based on the dideoxy-chain termination method (Sanger, et al., 1977). The nucleotide sequence was analyzed with the DNAsis program. A database search for homologous amino acid sequences was performed using the Blast algorithm at the NCBI via the Internet. The nucleotide sequence has been deposited in 30 GeneBank under accession number Z36940. Campylobacter chromosomal DNA digested by restriction enzymes were fractionated by electrophoresis in 0.7%

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agarose gel (0.8 mg DNA per lane), and DNA was transferred to GeneScreen Plus nylon membrane (DuPont-NEN) in 0.4 N NaOH solution for Southern hybridization. A 1.1 kb *jl*pA DNA fragment, generated by PCR amplification using primer P4 (5'-GAGAAACATATGAAAAAAGGTATTTTCTC-3') (SEQ. ID. NO.: 2) and primer RP7 (5'-AACTGCCGCCCATTAACATAGAAAAC-3') (SEQ. ID. NO.: 3), was employed as probe. Southern hybridization analysis was performed using the DIG (digoxigenin)-High Primer DNA Labeling and Chemiluminescent Detection kit (Boehringer Mannheim) according to the manufacturer's instructions.

Expression of JlpA in *E.coli*

Recombinant JlpA was expressed in *E.coli* using the T7 RNA polymerase / promoter system [19]. A 1,25 kb *jl*pA gene product was generated by PCR amplification using the primer P4. The PCR product containing artificial NdeI and BamHI site at each end was purified, digested with NdeI and BamHI, and ligated into pT7-7 vector. The resulting recombinant plasmid, pTlp33, contained a NdeI in frame fusion of T7 promoter [pT7] with *jl*pA gene at the start codon. To express the recombinant JlpA, pTlp33 was transformed into *E.coli* BL21[DE3]. The *E.coli* BL21 [DE3] [pTlp33] transformant was inoculated in M9 minimal medium containing 100 μ g ml⁻¹ Ap and inoculated at 37^o. Methionine assay medium was added at a final concentration of 10% when OD₆₀₀ = 0.1, and the culture was continued to grow to OD₆₀₀=0.2. The expression of JlpA was induced by 1mM IPTG. The culture was then divided into two aliquots. One of them was incubated at 37^oC for 30 min, followed by the incubation at 37^oC for 60 min with rifampicin [600 μ g ml⁻¹] for exclusive production of recombinant JlpA. The other one was incubated at 37^oC for 90 min as a control. [³⁵S] methionine [100Ci per ml of culture] was added in each aliquot, and incubated for 5 min. Cells were harvested by centrifugation and prepared for SDS-PAGE analysis. Radiolabelled proteins were resolved on a SDS-10% polyacrylamide gel, and visualized by auto radiography.

A 1.2 kb *jl*pA gene fragment without the first 16 amino acids coding sequence was PCR amplified and cloned into BamHI site of pGEX-2T.

The constructed plasmid, pXlp19, was transformed into *E.coli* JM101, and GST-JlpA fusion protein was expressed and purified according to the instructions provided by the supplier. The purified protein was used for raising antibodies.

Labelling JlpA with [³H]palmitate

5 *E.coli*BL21 was grown in M9 minimal medium containing Ap at 37°C. Methionine assay medium [10%] and methionine [20µg ml⁻¹] was added at OD₆₀₀=0.1. The culture was then grown to OD₆₀₀=0.2, and 1 mM IPTG was added. After incubation at 37°C for 30 min, [9, 10 [n] - ³H] palmitic acid was added, and followed by 3h incubation. *e.coli* cells were harvested and washed
10 twice in methanol, and the pellet was air dried. The cells were resuspended in PBS buffer, and lysed with the sample buffer. The released proteins were separated on a SDS-10% polyacrylamide gel, and radiolabelled lipoproteins were detected by autoradiography.

Antiserum

15 Polyclonal antiserum against JlpA was generated by immunizing a female New Zealand white rabbit. Purified GST-JlpA protein [500µg] was homogenized with complete Fread's adjuvant and used to immunize rabbit by intramuscular injection. Two boosters [250µg of the protein each] were given at 4 weeks and 6 weeks. Antiserum was harvested at 8 weeks
20 and stored at -20°C. Antiserum was affinity purified by using protein A sepharose 6MB as described by Harlow and Lane [20] when needed.

Electrophoresis and Western Blotting

Electrophoresis of SDS-PAGE was performed according to Laemmli [21]. Protein samples were boiled in SDS sample buffer for 10 min and
25 separated on SDS- 10% polyacrylamide gel. Proteins were then electroblotted onto a 0.2µm nitrocellulose membrane in a transfer buffer containing 25 mM Tris HCl pH8.3, 0.1% SDS, 0.2M glycine and 20% methanol for 3 hours at 60V. Membranes were incubated with 1:1000 diluted anti-JlpA antibodies and followed by anti-rabbit horseradish peroxidase-conjugated secondary antibody.
30 The immunoreactive proteins were visualized by chemiluminescence.

Extraction of outer membrane

Outer membrane proteins of *C.jejuni* TGH9011 were prepared using Sarcosyl extraction method [22] and Triton X-100 extraction method [23].

Extraction of cell surface proteins

5 The glycine-acid extraction method has been described to release surface protein antigens from *Campylobacter* [8,24,25]. *C.jejuni* cells harvested from five two-day old MH agar plates were washed twice with PBS buffer pH 7.4. The cell pellet was resuspended in 10 ml of .2 glycine-Hcl buffer pH2.2 and stirred at room temperature for 15 min before centrifugation at 10,000xg for 15 min. The
10 supernatant was neutralized with NaOH and then dialyzed against 3x1.5 L of PBS buffer. The cell pellet was washed once with PBS buffer and resuspended in 10 ml of PBS buffer.

Construction of isogenic *jlpA* mutants

15 An insertion mutant, *C. jejuni* TGH9011-JA1, and a deletion mutant, *C. jejuni* TGH9011-JA2, were constructed .

 In order to construct JA1, a plasmid pHDC1 was constructed by inserting a 1.45 kb kanamycin (Km) cassette derived from pILL550 (Labigne-Roussel et al., 1987) in pSK5 (Hani, 1997) into the XbaI site of *jlpA* gene in the plasmid pHDC.

20 In order to construct JA2, pHDC was digested with XbaI, and followed by Bal31 digestion at room temperature for 5, 10, 20, 30 min. The Bal31 digests were pooled, extracted with phenol/chloroform, precipitated with ethanol, ligated in the presence of XbaI linker (5'-GTCTAGAC-3') (SEQ. ID. NO.: 4), and transformed into *E. coli* DH5a. The transformants were screened for deletions by
25 colony PCR using primer P3 (5'-GTGTAAAAATGTAATTAATCACACAC-3') (SEQ. ID. NO.: 5) located upstream of putative promoter region and PR2 (5'-TTTGGATCCACTAGGGGAGAAT-3') (SEQ. ID. NO.: 6) located downstream of *jlpA*. The PCR products were examined for the presence of XbaI linker by XbaI digestion. One plasmid (pJLPD5), containing a XbaI linker and the biggest deletion
30 (confirmed by sequence analysis), was isolated, digested with XbaI, and ligated with Km cassette obtained from pSK5. The ligated DNA was transformed into *E. coli*

DH5a. The plasmid pJLPD5K with the insertion of the Km cassette in pJLPD5 was confirmed by digestion with different restriction enzymes, and used to generate a *jlpA* deletion mutant.

The plasmids pHDCK1 and pJLPD5K were introduced into *C. jejuni* TGH9011 by natural transformation (Wang and Taylor, 1990). Km resistant colonies were isolated from MH agar plates supplemented with Km (25 mg ml⁻¹). The presence of a disrupted *jlpA* gene with concomitant loss of the wild-type *jlpA* due to a double-crossover recombination event was verified by PCR analysis. One mutant obtained from pHDCK1 transformation was designated as *C. jejuni* TGH9011-JA1, and another mutant from pJLPD5K transformation was designated as *C. jejuni* TGH9011-JA2.

Adherence and invasion assays

HEp-2 cells (ATCC CCL23) were grown at 37°C in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS), 100 mg ml⁻¹ streptomycin, and 100 units ml⁻¹ penicillin G (Sigma) in a humidified 5% CO₂ incubator. Confluent HEp-2 monolayer was trypsinized, seeded into 24-well tissue culture plates at about 1×10⁵ cells per well in EMEM-10% FBS without antibiotics, incubated at 37°C for 18 h, and then washed twice with EMEM. The adherence and invasion assays were performed by co-incubating *jlpA* mutants JA1, JA2, and *C. jejuni* TGH9011 with HEp-2 cells, respectively, at a HEp-2 cell-bacteria ratio of about 1:100. The medium was removed after 2 h incubation, and monolayers were washed four times with EMEM, and then lysed with 0.1% (w/v) Triton X-100 at 37°C for 15 min. For the invasion assay, the monolayers were washed two times with EMEM after 2 h incubation, and then incubated for an additional 2 h with EMEM containing 100 mg ml⁻¹ gentamicin. The monolayers were washed two times with EMEM, and lysed with 0.1% (w/v) Triton X-100 for 15 min. The released bacteria were enumerated by plate counting on MH agar plates. Adherence efficiency was calculated as percentage of input bacteria adhering after extensive washing without gentamicin treatment (Yao et al., 1994). Invasion efficiency was

expressed as percentage of input bacteria surviving after gentamicin treatment (Oelschlaeger, et al., 1993).

EXAMPLE 1

Identification of the *jlpA* gene and properties of the gene product

5 A *hipO* gene encoding benzoylglycine amidohydrolase (hippuricase) was previously identified from *C. jejuni* TGH9011, and the nucleotide sequence of the *hipO* flanking region revealed the presence of seven other open reading frames (ORFs) in our laboratory (Hani and Chan, 1995). Analysis of the deduced amino acid sequences indicated that one of the ORFs, designated *orf14*,
10 encodes a putative lipoprotein. The *orf14* gene was named as *jlpA* (*jejuni* lipoprotein A).

 The 1116 bp *jlpA* gene encodes a polypeptide of 372 amino acids (aa) with a deduced molecular weight of 42.3kDa. The pSort algorithm analysis of the *jlpA* gene product revealed that the N-terminus of the molecule contained a 18-
15 amino-acid signal peptide with a central hydrophobic region. The sequence L-F-S-A-C (aa 14-18) at the distal end of the signal peptide closely matches the consensus processing site for lipid modification (Fig. 1). The G+C content of the *jlpA* coding region as well as flanking regions including *hipO* gene is 27.3%, which is slightly lower than that of *C. jejuni* genomic DNA (30-32% G+C) (Owen and Leaper, 1981;
20 Penner, 1988). A putative *s70* promoter, -35(TTTAAA) (SEQ. ID. NO.: 7) and-10(TATAAT) (SEQ. ID. NO.: 8)(Hawley and McClure, 1983), is located 31 nucleotides upstream of the translational initiation codon. The sequence AGGAGA (SEQ. ID. NO.: 9), a predicted site for ribosome binding, is located 5 nucleotides upstream of the methionine initiator codon (Fig. 1). A potential transcriptional termination
25 signal was found immediately downstream of the stop codon of *jlpA* gene.

 Two potential Fur-box sequences, FBSI and FBSII, were identified upstream of *jlpA* (Fig. 1). FBSI has 15 of 19 (with 7 of the 8 most conserved nucleotides) and FBSII has 13 of 19 (with 7 of the 8 most conserved nucleotides) nucleotides of the Fur-box consensus sequence (Thomas and Sparling, 1994). FBSI is
30 located 80 bp upstream of the putative promoter region,

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and FBSII is overlapped 6 bp by the -10 promoter element (Fig. 1). The presence of two Fur-box sequences on the 5' flanking region of *jl*pA suggests possible regulation by Fur.

EXAMPLE 2

5 Homology of JlpA with a rhoptry protein in malaria parasite

The BLAST search revealed that JlpA shared the highest homology with the rhoptry protein E8 of *Plasmodium yoelii*. E8, a huge protein containing 2401 amino acid residues, is a component of a club-shaped organelle called rhoptry which plays an important role in the recognition and invasion of *Plasmodium yoelii* to the host erythrocytes (Sinha et al., 1996). JlpA is homologous to two region of E8. JlpA (residues 22-361) shares 19% identity (41% similarity) with aa 509 to 877 of E8 (Fig. 2A) (SEQ. ID. NO.: 10), and JlpA (residues 93-349) has 25% identity (44% similarity) with E8 (residues 882-1114) (Fig. 2B) (SEQ. ID. NO. 11). In addition, both JlpA and E8 are hydrophilic and have similar hydrophobicity profiles at the homologous regions.

EXAMPLE 3

Expression and post-translational modification of the JlpA protein

The T7 RNA polymerase/promoter expression system (Tabor, 1990) was employed to determine the expression of JlpA in *Escherichia coli*. Two prominent proteins of 42 and 35 kDa, and several minor low molecular weight polypeptides were observed when *E. coli* BL21 (DE3) carrying pTlp33 was induced for synthesis of recombinant proteins in the presence of rifampicin (Fig. 3A). The 42 kDa polypeptide was close to the predicted size of the prolipoprotein (42.3 kDa) or mature JlpA molecule (40.5 kDa). The *jl*pA gene was the largest and the only translational ORF in the insert DNA, therefore, the 35 kDa polypeptide might be a degradation product of JlpA. The other minor low molecular weight plasmid-encoded proteins were also likely to be the result of proteolysis of the recombinant lipoprotein.

The lipoprotein nature of JlpA was confirmed by [³H]palmitate labeling of the recombinant protein. *E. coli* BL21 (DE3)/pTlp33 cells grown in the presence of [³H]palmitate were harvested and subjected to SDS-PAGE. A band of apparent

molecular mass of 41 kDa corresponding to recombinant JlpA was observed (Fig. 3B), indicating that the *C. jejuni* protein was lipid-modified in *E. coli*. A radiolabeled high molecular weight complex did not enter the SDS-polyacrylamide gel, suggesting that an insoluble lipoprotein aggregate present in the [3H]palmitate-labeled *E. coli* BL21 (DE3)/pTlp33 sample.

EXAMPLE 4

Surface localization of JlpA

To localize JlpA in the membrane of *C. jejuni* cells, sarcosyl extraction (Amako, et al., 1996) and Triton X-100 (Skare, et al., 1996) extraction methods were used for separating the inner and outer membrane proteins. The results from both methods showed that JlpA was found predominantly in the inner membrane fraction (data not shown). However, according to the +2 amino acid rule (Yamaguchi, et al., 1988), JlpA with a glycine at the +2 position is predicted to be located in outer membrane. Glycine-acid extraction method was used to further investigate the localization of JlpA in the membrane of *C. jejuni* TGH9011. Glycine-acid extracts from both *C. jejuni* TGH9011 and JA2 mutant cells were resolved by SDS-PAGE, and examined by immunoblotting with anti-JlpA antibodies. JlpA protein was found predominantly in glycine-acid extracts fraction from *C. jejuni* TGH9011, while no signal was found in the glycine-acid extracts from the JA2 mutant, indicating that JlpA was located on the surface of *C. jejuni* (Fig. 4A). However, the surface protein profiles of wild type and JA2 mutant (data not shown) showed no difference using Coomassie staining. Similarity of the two protein profiles possibly due to co-migration of JlpA with other proteins with similar molecular weight.

To confirm that JlpA is surface exposed, the susceptibility of JlpA on intact *C. jejuni* cells to proteinase K was determined. The result showed that JlpA of *C. jejuni* was completely digested when incubated with proteinase K (Fig. 4B), while FlgG, the flagellar basal rod protein with molecular weight of 28 kDa (Chan, et al., 1998), was not affected (Fig. 4C). Anti-FlgG antibodies also cross-reacted to a 92 kDa band which is possibly the hook protein FlgE (Fig. 4C). FlgG has high homology to FlgE (http://www.sanger.ac.uk/Projects/C_jejuni/).

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The 92 kDa band was digested by proteinase K, indicating a surface localization and consistent with a flagellar hook protein identity. Thus, the results obtained from the glycine-acid extraction and proteinase K digestion experiments indicated that JlpA is localized on the cell surface of *C. jejuni*. However, when
5 sarcosyl and Triton X-100 extraction methods were used, JlpA was found predominantly in the inner membrane fraction and very little was detected in the outer membrane fraction. Overall, the above findings suggest that JlpA is loosely associated with the cell surface.

EXAMPLE 5

10 **Release of JlpA to culture medium during growth**

It has been reported that some Gram-negative pathogens release lipoproteins during bacterial growth, and released lipoproteins play an important role in the induction of cytokine production and/or pathology associated with Gram-negative bacterial infections (Zhang, et al. 1998). The fact
15 that JlpA is a surface exposed lipoprotein prompted us to investigate if JlpA is released to the culture medium during the bacterial growth. *C. jejuni* TGH9011 was grown in MH broth at 37°C in CO₂ incubator with shaking and sampled at various intervals. Release of JlpA was examined by immunoblotting with anti-JlpA antibodies. As shown in Fig.5, JlpA was detectable in bacterial culture
20 supernatant of all stages, and its concentration increased in a time-dependent fashion.

EXAMPLE 6

Role of jlpA in the adherence to HEp-2 cells

Two isogenic jlpA mutants, *C. jejuni* TGH9011-JA1 (JA1) and
25 TGH9011-JA2 (JA2) (Fig. 6), were used to examine the role of jlpA in the adherence and invasion to HEp-2 cells in vitro. *C. jejuni* JA1 mutant was constructed by inserting a kanamycin resistant gene cassette (Labigne-Roussel et al., 1987) into a unique XbaI site within the jlpA coding sequence. *C. jejuni* JA2 is a deletion mutant, constructed by Bal 31 digestion followed by insertion with a
30 kanamycin cassette. Sequence analysis showed that the JlpA N-terminal coding region including the -35 element of the jlpA promoter was deleted, and only 5

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C-terminal amino acid residues encoding sequence was left intact (Fig. 6). Immunoblotting analysis using antibodies against JlpA showed that no immunoreactive JlpA band can be detected in either JA1 or JA2 mutant (data not shown). No detectable difference in growth was observed among parental C. jejuni TGH9011 and the two mutant strains when cultured in either MH or MEM medium (data not shown).

The ability of C. jejuni TGH9011 and two mutants, JA1 and JA2, to adhere and invade HEp-2 cells was examined. The adherence activity of JA1 and JA2 to HEp-2 cells was reduced to 19.4% and 18.0% of the wild type strain, respectively (Table 1). The invasion was also reduced, which might be due to the reduction of adherence (data not shown).

EXAMPLE 7

Adherence Inhibition of C. jejuni by antibodies against JlpA

To further investigate whether JlpA plays a role in adherence directly, polyclonal antibodies raised against purified JlpA were used. The affinity-purified anti-JlpA antibodies inhibited the adherence of C. jejuni TGH9011 to HEp-2 cells by 68% (Table 2). The lower level of adherence of JA2 was not further reduced by anti-JlpA antibodies (data not shown). Control preimmunized serum did not inhibit the adherence of TGH9011 to HEp-2 cells (data not shown).

EXAMPLE 8

Inhibition of C. jejuni adherence by purified recombinant JlpA

Purified JlpA protein was used to examine its effect on adherence of C. jejuni to HEp-2 cells. The adherence of C. jejuni TGH9011 to HEp-2 cells was inhibited 83% by co-incubating HEp-2 cells with GST-JlpA (5mg/ml), while GST (5 mg/ml) only did not affect the adherence of C. jejuni (Table 2). The binding of JlpA to HEp-2 cells was further confirmed by immunoblotting analysis with antibodies against GST-JlpA (Fig. 7). A 68 kDa GST-JlpA band was observed in the GST-JlpA bound HEp-2 cell lysate. No signal was detected from HEp-2 cell lysate and GST bound HEp-2 cell lysate.

EXAMPLE 9

Presence of *jlpA* in *C. jejuni* strains

To determine whether the *jlpA* gene is specific to *C. jejuni* species, *jlpA*-specific primers P4 and PR7 were used to amplify the gene from eight *C. jejuni* strains and several other *Campylobacter* species, such as *C. coli*, *C. lari*, *C. sputorum*, and *C. upsaliensis*. A 1.1 kb PCR products was observed in all eight *C. jejuni* strains, isolated from patients or environment and no detectable amplicons were observed from the other *Campylobacter* species (data not shown). Southern hybridization analysis was used to further confirm the absence of *jlpA* homologous sequence in those *Campylobacter* species. As shown in Fig. 8A, the *jlpA* DNA probe which hybridized to a 2.2 kb band of *Cla*I digested genomic DNA of *C. jejuni* TGH9011, did not hybridize with the genomic DNA digests from the other *Campylobacter* species. Thus, *jlpA* gene is specific to *C. jejuni*. Expression of JlpA in *C. jejuni* strains were clarified by immunoblotting (Fig. 8B).

DISCUSSION OF EXAMPLES

C. jejuni surface proteins play an essential role in the bacterial adherence to mammalian cells, and this ligand-receptor interaction are mediated by adhesins on the bacterial surface. Two adhesins, PEB1 and CadF, have been characterized in *C. jejuni* (Pei, et al., 1998; Konkel, et al., 1997), and both are outer membrane lipoproteins. PEB1 is a homologue of a component of ABC transport system in Gram-negative bacteria, and CadF is a peptidoglycan-associated protein. In this study, we identified a novel *C. jejuni*-unique gene, *jlpA*, which encodes a surface exposed lipoprotein (JlpA) that binds to HEp-2 cells and is released to the culture medium during growth. JlpA plays an important role in the adherence of *C. jejuni* to HEp-2 cells.

Sequence analysis showed that JlpA shares homology with the E8 rhoptry protein of rodent malaria parasite, *P. yoelii* (Sinha, et al., 1996). The E8 protein, a member of a multigene family proteins with a relative molecular mass of 235 kDa, is a component of a club-shaped organelle called rhoptry. These proteins recognize the subset of erythrocytes responsible for parasite invasion (Holder and Freeman, 1981; Freeman et al., 1980), and are

associated with generating clonal phenotypic variation in rodent malaria (Preiser, et al., 1999). JlpA is the first protein reported in *Campylobacter* that shares homology with eukaryotic proteins. The G+C content (27.3%) of the jlpA coding region is lower than that of the complete genome of *C. jejuni* (30-32%
5 G+C) (Owen and Leaper, 1981; Penner, 1988), suggesting a possible acquisition through horizontal transfer from a eukaryotic genome with a low G+C content, such as malaria parasite. *P. falciparum* has a G+C content of 18-20% (<http://www.sanger.ac.uk/Projects/Protozoa>). Furthermore, the hipO-jlpA is located in a 15 kb region specific to *C. jejuni* (Chan et al., 2000), indicating that
10 the region was acquired since the split of *C. jejuni* and *C. coli*.

The ability to incorporate [3H]palmitate, the hydrophobic nature of the N-terminal amino acid sequence, and the presence of a lipobox sequence L-F-S-A-C (Hayashi and Wu, 1990; Wu, 1996), suggest strongly that JlpA is a lipoprotein. The low level of [3H]palmitate incorporated may be due to
15 the low expression as well as degradation of JlpA in *E. coli*. Additionally, the expression of JlpA may be harmful to *E. coli* cells since a similar problem has been reported by Gomez et al. (1994).

In Gram-negative bacteria, prolipoprotein are synthesized in the cytoplasm. The signal sequence targets the prolipoprotein to the cytoplasmic
20 membrane. The lipobox is recognized by the cytoplasmic membrane prolipoprotein modification and processing enzymes that lead to the formation of N-acyl-diacylglycerylcystein, a lipid-modified structure at the N-terminus of the polypeptide (Hayashi and Wu, 1990; Wu, 1996). The mature lipoprotein is then transported to its final destination at the outer membrane or retained at
25 the cytoplasmic membrane. The glycine-acid extraction and proteinase K digestion experiments indicated that JlpA is a surface-exposed protein in *C. jejuni*. We proposed that the identification of JlpA to the inner membrane fraction by the sarcosyl and Triton X-100 methods may be attributed to a loose association of JlpA to the outer membrane and thereby released to the inner
30 membrane fraction by the detergents. Trust and Logan (1984) reported a loosely associated surface protein of *C. jejuni* which was also fractionated to the inner

membrane fraction by the sarcosyl extraction method. A hydrophobicity calculation by the method of Kyte & Doolittle identified a potential hydrophobic transmembrane domain (aa 353-369) at the C-terminus of JlpA. We proposed that the lipidated N-terminus and the hydrophobic C-terminus of JlpA are
5 embedded in the outer membrane while the central hydrophilic region is exposed on the bacterial surface.

It has been reported that some enteric pathogens, such as *E. coli*, *Salmonella typhimurium*, and *Yersinia enterocolitica*, release lipoproteins during growth (Zhang, et al. 1998). The released lipoproteins may have a role in
10 the pathogenesis of bacterial infection because lipoproteins have been shown to induce the formation of tumor necrosis factor alpha and the release of interleukin-6 (IL-6) by macrophages (Zhang, et al., 1997; 1996). JlpA is released to the culture medium by *C. jejuni*. The function of the released JlpA remains to be established. The mechanism by which lipoproteins are released from bacteria
15 during growth is still unclear. Gram-negative bacteria are known to release membrane fragments to the surrounding milieu during growth (Wensink and Witholt, 1981). *C. jejuni* has been reported to form blebs consisting of outer membrane fragments. These blebs are constantly released into the medium during growing (Pead, 1979; Logan and Trust, 1982). It is possible that the release
20 of JlpA is associated with the release of outer membrane fragments and formation of blebs.

Both insertion mutant JA1 and deletion mutant JA2 of *jlpa* showed significant reduction in adherence to HEp-2 cells. The adherence of *C. jejuni* to HEp-2 cells was reduced by both affinity-purified anti-JlpA antibodies
25 and purified JlpA. In addition, purified JlpA binds to HEp-2 cells. The above findings suggest that JlpA is an adhesin and has a direct role in the adherence of *C. jejuni* to HEp-2 cells. It also indicates that JlpA is a good potential candidate for development of a vaccine against *C. jejuni* infection.

30 All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each

- 36 -

individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE SPECIFICATION

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20 DETAILED FIGURE LEGENDS

Figure 1. The 5' nucleotide sequence and the N-terminus deduced amino acid sequence of the *jlplA*. Deduced transformational product was shown by using single-letter amino acid residue codes. A consensus recognition sequence for signal peptidase II was shown in boldface and the predicted cleavage site was
 25 marked by a vertical arrow. The putative ribosome binding site [RBS] and putative σ^{70} promoter sequence [135 and -10] were underlined. Two potential Fur-binding sequences, FBSI and FBSII, were overlined.

Figure 2. The alignment shown is modified from the alignment obtained from a
 30 BlastP database search.

Figure 3. Expression of recombinant JlpA in *E.coli*. *E.coli* BL21[DE3] carrying pT7-7 vector or pTlp33 was induced to synthesize the recombinant proteins with IPTG. Radioactive methionine or palmitate was added to label JlpA. Total cellular proteins were separated on a SDS-10% polyacrylamide gel and radiolabelled polypeptides were visualized by autoradiography. [A] [³⁵S] methionine-labelled proteins obtained in the absence of presence of rifampicin. [B] [³H] palmitate-labelled lipoproteins.

Figure 4. Surface localization of JlpA in *C.jejuni* TGH9011. [A] Glycine-acid extraction fraction from wild type *C.jejuni* TGH9011 and mutant JA2 were applied to a SDS-10% PAGE, immunoblotted onto a nitrocellulose membrane and probed with anti-JlpA antibodies. Lanes: 1, 4, untreated whole-cell lysate [total proteins]; 2,5, glycine-acid treated whole-cell lysate; 3,6, glycine-acid extracted proteins. Lanes 1,2,3: proteins from wild type; 4,5,6: proteins from JA2 mutant. [B] Surface proteolysis of intact *C.jejuni* TGH9011 cells. *C.jejuni* cells were collected from MH broth, washed with distilled water and resuspended in water at a concentration of 5×10^8 cells ml⁻¹. An aliquot of 50 µl cells in each tube were exposed to different amount of proteinase K at 37°C for 15 min. Samples were analyzed by immunoblotting and probed with anti-JlpA antibodies. Lanes:1, No proteinase K [control]; 2, 0.1 µg; 3, 0.5 µg; 4, 2.5 µg; 5, 10µg of proteinase K; [C] Same sample as in panel [B] but probed with anti-FlgG antibodies.

Figure 5. Release of JlpA during growth. *C.jejuni* TGH9011 was grown in MH broth medium at 37°C with shaking in the atmosphere of 5% CO₂ and 95% air. Samples were withdrawn at the time points indicated. Cells were pelleted and lysed with the sample buffer. Culture supernatants were filtered through 0.22-µm syringe filters and precipitated with TCA. The same OD₆₀₀ equivalent of cell-associated proteins and the same ten-fold TCA-concentrated OD₆₀₀ equivalent of culture medium proteins were separated on a SDS-10% polyacrylamide gel. Both

cell proteins and supernatant proteins were transformed onto the same nitrocellulose membrane followed by immunoblotting with anti-JlpA antibodies.

- 5 Figure 6. Generic organization of hipO-jlpA region and construction of jlpA mutants. The jlpA insertion mutant, *C.jejuni* TGH9011-JA1, was constructed by inserting kanamycin cassette into the XbaI site of the jlpA gene. Deletion mutant of jlpA, *C.jejuni* TGH9011-JA2, was constructed by inserting a kanamycin cassette into a Bal31 digested jlpA gene.

Figure 7. Specific binding of purified JlpA to HEp-2 cells. Confluent HEp-2 monolayers in a 24-well plate were washed twice with EMEM. One ml EMEM containing 10 µg purified GST-JlpA or GST only as control was added onto the HEp-2 monolayer. The plate was incubated at 37°C for 2 h in the atmosphere of
 15 5% CO₂ and 95% air. The HEp-2 monolayers were washed 6 times with EMEM and then lysed with 150 µl of 10mM Tris.HCl buffer pH 7.4 containing 0.8% SDS [33]. Protein samples were subjected to SDS-10% polyacrylamide gel, followed by immunoblotting with anti-GST-JlpA antibodies. Lanes:1, HEp-2 cells; 2, HEp-2 binded with GST proteins; 3, HEp-2 cells binded with GST-JlpA proteins.

Figure 8. The presence of jlpA in *Campylobacter* species. [A] Southern hybridization analysis of *Campylobacter* DNAs with a jlpA gene probe. A 1.1 kb DIG-labelled jlpA gene fragment was used to probe ClaI-digested genomic DNAs. Lanes: 1, *C.jejuni* TGH9011; 2, *C.coli* ATCC 33559^T; 3, *C.coli* ATCC 43482;
 25 4, *C.coli* ATCC 43486; 5, *C.coli* ATCC 49299; 6, *C.lari* ATCC 35221^T; 7, *C.sputorum* subsp. *bubulus* ATCC 33562^T; 8, *C.upsaliensis* ATCC 43954^T. [B] Immunoblotting of the whole cell lysates from different *C.jejuni* strains with anti-JlpA antibodies. Lanes: 1, TGH9011; 2, 81-176; 3, CEPA-3C; 4, OH4382; 5, OH4384; 6, LCDC-13267; 7, CA21-106; 8, 1-5R.

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Table 1. Adherence of *C. jejuni* TGH9011 and its *jlpA* mutants to HEp-2 cells.

Strain	% adherence	% wild type
TGH9011	0.35 ± 0.14	100
TGH9011-JA1	0.068 ± 0.03	19.4
TGH9011-JA2	0.063 ± 0.02	18.0

Results represent the mean of four independent experiments $\pm$ standard
5 deviation.

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Table 2. Inhibition of adherence by affinity-purified anti-JlpA antibodies (α -JlpA) or purified GST-JlpA protein.

Treated cell	Addition	% inhibition
C. jejuni TGH9011	PBS	0
	α -JlpA	68
HEp-2 cells	PBS	0
	GST	0
	GST-JlpA	0
		83

- 5 C.jejuni TGH9011 cells were incubated with affinity-purified anti-JlpA at 37°C for 30 min, and then washed with PBS buffer before added to HEp-2 monolayer. Prior to adding C. jejuni TGH9011 cells, HEp-2 monolayer was incubated with GST-JlpA or GST only at 37°C for 30 min and washed twice with EMEM.

Table 3. Strains and plasmids used in this study.

Strain or plasmid Relevant characteristics Source or reference

C. jejuni

5	TGH9011(ATCC43431)	Serotype O:3, clinical isolate
	81-176	Clinical isolate
	CEPA-3C	Clinical isolate from Guillain-Barre Syndrome patient
	OH4382	Clinical isolate from Guillain-Barre Syndrome patient
	OH4384	Clinical isolate from Guillain-Barre Syndrome patient
10	LCDC-13267	Environmental isolate
	CA21-106	Environmental isolate
	1-5R	Environmental isolate
15	J. L. Penner	
	M. J. Blaser	
	G. A. Clark	
	J. L. Penner	
	J. L. Penner	
20	J. Odumeru	
	J. Odumeru	
	J. Odumeru	
	<i>C. coli</i>	
	ATCC 33559	
25	ATCC 43482	
	ATCC 43486	
	ATCC 49299	
	Type strain of the species	
	Serotype reference strain for O:46	
30	Serotype reference strain for O:51	
	Serotype reference strain for O:61	

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J. L. Penner

J. L. Penner

J. L. Penner

J. L. Penner

5 C. lari

ATCC 35221

Type strain of the species

ATCCa

C. sputorum subsp. Bubulus

10 ATCC 33562

Type strain of the species

ATCC

15 C. upsaliensis

ATCC 43954

Type strain of the species

ATCC

E. coli

20 DH5a

JM101

BL21(DE3)

25 F- f80dlacZDM15 recA1 endA1 hsdR17 gal- gyrA96

supE thi D(lac-proAB) F' [traD36 proAB+ lacIq lacZDM15]

hsdS gal (lcIts857 ind1 Sam7 nin5 lacUV5-T7 gene 1)

BRLb

30 Jenkinson, 1994

Studier, 1990

Plasmids

- pBR322
- pBluescriptII KS
- 5 pGEX-2T
- pILL550

- pSK5
- pHIP-O

- pHDC
- pHDCK1
- pJLPD5
- pJLPD5K
- 15 pT7-7
- pTlp33
- pXlp19
- AmpR, TetR, general cloning vector
- AmpR, lacZ, cloning vector
- 20 AmpR, glutathione S-transferase (GST) gene fusion vector
- AmpR, KmR, E. coli-C. jejuni shuttle vector

- AmpR, KmR, 1.45 kb KmR cassette of pILL550 in pUC19
- A pBR322 clone containing hipO-jlpA DNA fragment from C. jejuni DNA
- 25 library
- pHIP-O deletion derivative carrying jlpA by ClaI digestion
- pHDC derivative carrying jlpA::KmR insertion
- pHDC derivative carrying Bal31 deleted jlpA and a XbaI linker
- pJLPD5 derivative carrying DjlpA::KmR insertion
- 30 AmpR, gene expression vector by T7 RNA polymerase
- pT7-7 derivative carrying jlpA

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pGEX-2T derivative carrying jlpA

Bolivar, 1977

Stratagene

Pharmacia

5 Labigne-Roussel, 1990

Hani, 1997

Hani and Chan, 1995

This study

This study

10 This study

This study

Tabor, 1990

This study

This study

15 a ATCC, American Type Culture Collection; b BRL, Bethesda Research
Laboratories.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Joe, Angela
Jin, Songmu
Chan, Voon Loong
- (ii) TITLE OF INVENTION: A Surface-Exposed Lipoprotein to
Campylobacter Jejuni Involved in Adherence to HEp-2 Cells
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
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 - (C) CITY: Toronto
 - (D) STATE: Ontario
 - (E) COUNTRY: Canada
 - (F) ZIP: M5H 3Y2
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: CA 2,296,869
 - (B) FILING DATE: 24-JAN-2000
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Gravelle, Micheline
 - (B) REGISTRATION NUMBER: 2800
 - (C) REFERENCE/DOCKET NUMBER: 2223-92
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 416-364-7311
 - (B) TELEFAX: 416-361-1398

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 300 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGCTTAAAC CTGTTGCAAA AATATATAAT AATTGCAAAG TTAATTATAC TTAAAATTAT	60
AAATAATCCA TTTCAAAGAA ACTAAAAAGA TTTTCCTATG TGTA AAAAATG TAATTAATCA	120
CACACTCTCT TTTAGTATTG TTAAATTTT AAGTAAAAAA AGATATAATC TTTTTTTTAA	180

-50-

ATTTTTTAAG GAGAAACTA TGAAAAAAGG TATTTTCTC TCTATTGGAA TAGCTGTTTT 240
GTTTTCAGCT TGCGGAAATT CCATAGATGA AAAACAGTT AAAAAATATG AAAATCAACT 300

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAGAAACATA TGAAAAAAGG TATTTTCTC 30

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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

AACTGCCGCC CATTAACATA GAAAAC 26

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 8 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTCTAGAC 8

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

-51-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
GTGTAAAAAT GTAATTAATC ACACAC 26

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
TTTGGATCCA CTAGGGGAGA AT 22

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
TTTAAA 6

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
TATAAT 6

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGAGA

6

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 340 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ile	Asp	Glu	Lys	Thr	Val	Lys	Lys	Tyr	Glu	Asn	Gln	Leu	Asn	Gln	Thr	1	5	10	15
Val	Lys	Gln	Glu	Ile	Ala	Ser	Leu	Ser	Gln	Asp	Ser	Gly	Ile	Lys	Ile	20	25	30	
Glu	Phe	Ser	Asp	Phe	Lys	Cys	Asn	Ala	Asp	Gly	Asp	Phe	Ile	Ala	Cys	35	40	45	
Leu	Ser	Pro	Asn	Phe	Lys	Thr	Leu	Ala	Lys	Asp	Asn	Asn	Asn	Glu	Tyr	50	55	60	
Gln	Glu	Leu	Phe	Gln	Ala	Lys	Asn	Ile	Lys	Ile	Arg	Ser	Asn	Glu	Ile	65	70	75	80
Tyr	Lys	Gly	Glu	Ala	Asn	Ala	Ser	Ile	Ser	Ile	Lys	Glu	Tyr	Tyr	Asn	85	90	95	
Asp	Leu	Phe	Lys	Asn	Gln	Lys	Ser	Ile	Gln	Ser	Asn	Leu	Val	Phe	Glu	100	105	110	
Asp	Phe	Lys	Leu	Gly	Glu	Lys	Val	Val	Ser	Asp	Ile	Asn	Ala	Ser	Leu	115	120	125	
Phe	Gln	Gln	Asp	Pro	Lys	Ile	Arg	Ser	Phe	Ile	Asn	Lys	Leu	Ser	Ser	130	135	140	
Asp	Ser	Tyr	Thr	Leu	Ser	Phe	Asp	Asn	Ser	Ile	Asn	Lys	Gln	Glu	Asn	145	150	155	160
Asn	Tyr	Leu	Asp	Asn	Leu	Asp	Ile	Lys	Phe	Tyr	Asn	Ala	Lys	Leu	Asn	165	170	175	
Phe	Asn	Thr	Asn	Leu	Asn	Ile	Asn	Leu	Lys	Glu	Asp	Leu	Leu	Asn	Tyr	180	185	190	
Leu	Asp	Ser	Lys	Gly	Ile	Lys	Phe	Asn	Thr	Gln	Thr	Leu	Ala	Met	Asp	195	200	205	
Glu	Gln	Ala	Ile	Asn	Glu	Leu	Leu	Asn	Ile	Ala	Asn	Tyr	Glu	Gln	Ala	210	215	220	
Ser	Asp	Phe	Ser	Asn	Thr	Ile	Gln	Lys	Tyr	Ile	Ile	Leu	Asn	Asn	Phe	225	230	235	240

(2) INFORMATION FOR SEQ ID NO:11:

(A) LENGTH: 257 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Asn 1	Ile	Lys	Ile	Arg 5	Ser	Asn	Glu	Ile	Tyr 10	Lys	Gly	Glu	Ala	Asn 15	Ala
Ser	Ile	Ser	Ile 20	Lys	Glu	Tyr	Tyr	Asn 25	Asp	Leu	Phe	Lys	Asn 30	Gln	Lys
Ser	Ile	Gln 35	Ser	Asn	Leu	Val	Phe 40	Glu	Asp	Phe	Lys	Leu 45	Gly	Glu	Lys
Val 50	Val	Ser	Asp	Ile	Asn	Ala 55	Ser	Leu	Phe	Gln	Gln 60	Asp	Pro	Lys	Ile
Arg 65	Ser	Phe	Ile	Asn	Lys 70	Leu	Ser	Ser	Asp	Ser 75	Tyr	Thr	Leu	Ser	Phe 80
Asp	Asn	Ser	Ile	Asn 85	Lys	Gln	Glu	Asn	Asn 90	Tyr	Leu	Asp	Asn	Leu 95	Asp
Ile	Lys	Phe	Tyr 100	Asn	Ala	Lys	Leu	Asn 105	Phe	Asn	Thr	Asn	Leu 110	Asn	Ile
Asn	Leu	Lys 115	Glu	Asp	Leu	Leu	Asn 120	Tyr	Leu	Asp	Ser	Lys 125	Gly	Ile	Lys
Phe	Asn 130	Thr	Gln	Thr	Leu	Ala 135	Met	Asp	Glu	Gln	Ala 140	Ile	Asn	Glu	Leu
Leu 145	Asn	Ile	Ala	Asn	Tyr 150	Glu	Gln	Ala	Ser	Asp 155	Phe	Ser	Asn	Thr	Ile 160

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Gln	Lys	Tyr	Ile	Ile	Leu	Asn	Asn	Phe	Lys	Ile	Asp	Ser	Thr	Leu	Lys
				165					170					175	
Thr	Glu	Gly	Val	Phe	Ser	Ser	Tyr	Ile	Thr	Thr	Ala	Lys	Glu	Asn	Leu
			180					185					190		
Gln	Thr	Leu	Lys	Thr	Gln	Ser	Gln	Asn	Glu	Glu	Gln	Ala	Leu	Ile	Phe
		195					200					205			
Asp	Lys	Ala	Leu	Ala	Ile	Leu	Asn	Asn	Ile	Thr	Gln	Asn	Asp	Asp	Tyr
	210					215					220				
Lys	Leu	Asn	Leu	Asp	Leu	Lys	Phe	Lys	Asn	Ile	Pro	Val	Ser	Asp	Tyr
225					230					235					240
Ser	Thr	Gln	Gly	Ile	Asp	Ser	Ile	Glu	Lys	Leu	Ser	Ile	Asn	Asn	Gln
				245					250					255	

Asp

The Embodiments of the Invention in which an exclusive property or privilege is claimed are defined as follows:

- 5 1. A purified and isolated nucleic acid molecule having a sequence encoding a protein associated with adherence and invasion of *Campylobacter jejuni*.
2. A purified and isolated nucleic acid molecule as claimed in claim 1, which comprises:
 - 10 [a] a nucleic acid sequence as shown in Figure 1;
 - [b] nucleic acid sequence complementary to [a];
 - [c] nucleic acid sequence which are at least 70% homologous to [a]; or
 - [d] a fragment of [a] or [b] that is at least 15 bases and which will
 - 15 hybridize to [a] or [b] under stringent hybridization conditions.
3. A recombinant molecule adapted for transformation of a host cell comprising a nucleic acid molecule as claimed in claim 2 and expression control sequence operatively linked to the DNA segment.
4. A transformant host cell including a recombinant molecule as claimed in claim 3.
5. A method for preparing a JplA protein associated with
25 adherence or invasion of *C. jejuni* utilizing a purified and isolated nucleic acid molecule as claimed in claim 1.
6. A purified and isolated polypeptide having the amino acid sequence of JlpA.
7. A purified and isolated polypeptide as claimed in claim 6 having the amino acid sequence in Figure 1 with a lipid moiety.

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8. A protein encoded by the purified and isolated nucleic acid molecule as claimed in claim 2.
9. A monoclonal or polyclonal antibody specific for an epitope of
5 the purified and isolated polypeptide as claimed in claim 6.
10. A monoclonal or polyclonal antibody for an epitope of the purified and isolated polypeptide as claimed in claim 7.
- 10 11. An antibody as claimed in claim 9 that binds a distinct epitope in an unconserved region of the polypeptide.
12. An antibody as claimed in claim 10 that binds a distinct epitope unconserved region of the polypeptide.
13. A method for detecting *C.jejuni* in a sample comprising contacting a sample with a monoclonal or polyclonal antibody as claimed in claim 9 that is capable of being detected after it becomes bound to JplA protein in a sample.
- 20 14. A method for detecting *C.jejuni* in a sample comprising contacting a sample with a monoclonal or polyclonal antibody as claimed in claim 10 that is capable of being detected after it becomes bound to JplA protein in a sample.
- 25 15. A method for detecting the presence of a nucleic acid molecule as claimed in claim 2 in a sample, comprising:
[a] contacting the sample with a nucleotide probe capable of hybridizing with the nucleic molecule, to form a hybridization product, under conditions which permit the formation of hybridization product; and
30 [b] assaying for the hybridization product.

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16. A method for detecting the presence of a nucleic acid molecule as claimed in claim 2, or a predetermined oligonucleotide fragment thereof in a sample, comprising:
- [a] treating the sample with primers which are capable of amplifying the nucleic acid molecule or the predetermined nucleotide fragment thereof in a polymerase chain reaction to form the amplified sequences; and
 - [b] assaying for amplified sequences.
17. A kit for detecting *Campylobacter jejuni* by assaying for a JlpA protein in a sample comprising:
- [a] a monoclonal or polyclonal antibody as claimed in claim 9;
 - [b] reagents required for binding of the antibody to JlpA protein in the sample; and
 - [c] directions for use.
18. A kit for detecting the presence of nucleic acid molecule as claimed in claim 2 in a sample comprising:
- [a] a nucleotide probe capable of hybridizing with the nucleic acid molecule;
 - [b] reagents required for hybridization of the nucleotide probe with the nucleic acid molecule; and
 - [c] directions for use.
19. A kit for detecting the presence of a lipoprotein or a lipid moiety in a sample on a polypeptide as claimed in claim 7 and directions for use.
20. A method of inhibit the binding of *C. jejuni* comprising administering to an animal an effective amount of a substance capable of inhibiting the adherence of a JlpA protein in an animal in need thereof.

FIGURE 1

A (Identities = 75/383(19%), Positives = 161/383(42%), Gaps = 57/383(14%))

Jlpa 22 IDEKTVKKYENQLNQT-----VKQEIASLSQDSGIKIEFSDF--KCNADGDFIA-C--- 69
 + +- +KKYEN+ N + K+ I Q+ + D+ C + I C
 E8 509 VSDEEIKKYENKFNDKSLINETKKSIEEEYQNINTLKKVDDYIKVCLNTNELITNCHNK 568

Jlpa 70 -----LSPNFKTLAKDNN-----NEYQELFQAKNIKIRSNEIYKGEANASISIKE-- 114
 L+ N KT+ + N+ +++++ + K ++ + N + KE
 E8 569 QTTLKDKLNQNIKTIKETNSIDKIYTDKFENILTDRKTELETFTGLSLNNHESNNKELL 628

Jlpa 115 -YYNDLFKNQKSIQSNLVFEDFKLGEKVVSDINASLFQQDPKIRSFINKLSSDSYTLSD 173
 Y+ DL N + N++++ F EK V DI +++ I -- + YT +
 E8 629 TYFYDLKANLGKNKENMLYKQFNEKEKAVEDIK----KKNVDINKIYSNIEITITYSIY- 683

Jlpa 174 NSINKQENNYLDNLDIKFYNAKLNFTNLNI-NLKE-----LLNYLDSKGIF-NT 223
 +IN+ N + I+ N K+ N+ NL E D +- K IK+ +
 E8 684 -NINEDTENEIGK-SIELLNTKVLEKVKANVTNLNEIKEKLDYDFQDFGKEKNIKYPDE 741

Jlpa 224 QTLAMDEQAINELLNIANYEQASDFSNTIQKYIILNNFKIDSTLKTGVFSSYITTAKEN 283
 + D +N+ ++ + E ++ + -I +ID K T E+
 E8 742 NKIKNDIDTLNQKID-KSIETLTEIKKNSENHIDEIKGQIDKLKKVPNK-----TMFNED 795

Jlpa 284 LQTLKTQSQN-----EEQALIFDKALAILNNITQNDYKLNLDLKFKNIPVSDYSTQGID 338
 + +- + -N +++ I+ + -LN I++ ++ K -L- K KNI +S + G
 E8 796 PKEIEKKIENIVEKIDKKKNYKEIDKLLNEISKIENDKTSLE-KLKNINLSYGKSLGNL 854

Jlpa 339 SIEKLSINNQDATEVLKIILPFI 361
 +++++ + A +K + +I
 E8 855 FLQQIDEKKKAHTIKAMEAYI 877

B (Identities = 69/269(25%), Positives = 122/269(44%), Gaps = 48/269(17%))

Jlpa 93 NIKIRSNEIYKGEANASISIKEYYNDLFKNQKSIQSNLVFEDFKL-----GEKVVSDI 145
 NIK +S EI K E N ++ IK D+ K K++ N+ +D+K+ E+ +SDI
 E8 882 NIKKKSQEIEK-EMNINMDIKM---DIHKEMKAL--NISHDDYKIYHTTSKNHEEKISDI 935

Jlpa 146 --NASLFQQDPKIRSFINKLSSDSYTLSDNSINKQE--NNYLDNLDIKFYNAKLNFTNL 202
 N+ QD S+IN + + ++ N + N YL ++ + KLN +
 E8 936 RKNSLKIIQDFSEESYINDIKKELEKNVLESQNNNTDINQYLSKIENIYNILKLNKIKKI 995

Jlpa 203 NINLKEDLLNYLDSKGIFNTQTLAMDEQAINELLNIANYEQASDFSNTIQKYIILNNFK 262
 +KE + ++ K N + L+ E+ I +L ++ ++ K
 E8 996 IDKVKE-YTDEIEKNNKKINAE-LSNSEKIITQLKENSSSLKECQS-----K 1039

Jlpa 263 IDSTLKTGVFSSYITTAKENLQTLKTQSQNEEQALIFDKALAILNNITQN-DDYKLNLD 321
 I ST+ +Y++ +N+ LKT NE+ + N +N ++Y N+
 E8 1040 IKSTID-----DNYVSECIKNITNLKTYIVNEKNNI-----NTYFKNAEEYNQNV 1085

Jlpa 322 LKFKNIPVSDYSTQGIDSIEKLS-INNQD 349
 L F NI ++D +Q I +I+K + NN D
 E8 1086 LNFNNIEMADTKSQYILNIKKNNGTNNTD 1114

FIGURE 2

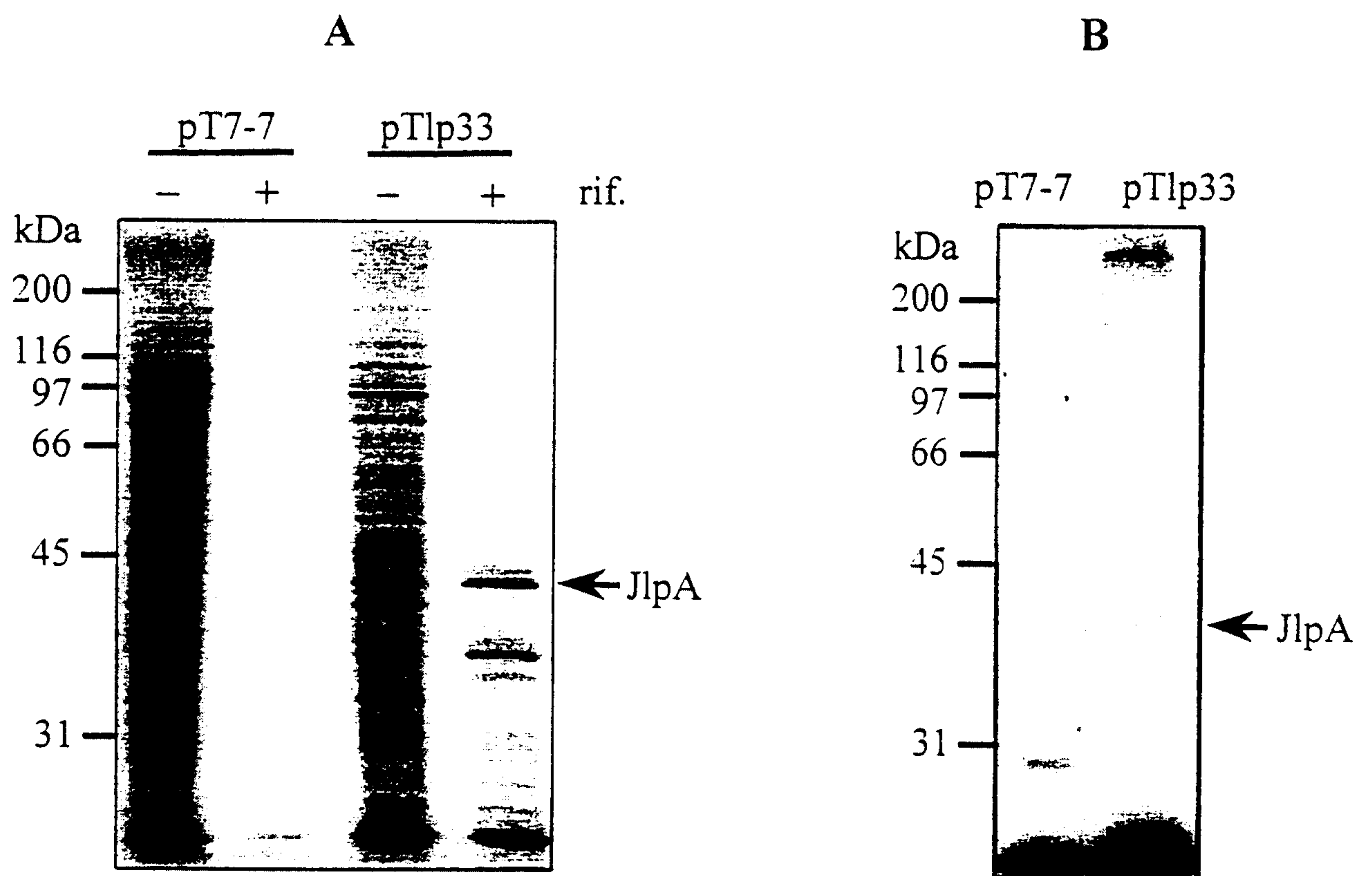


FIGURE 3

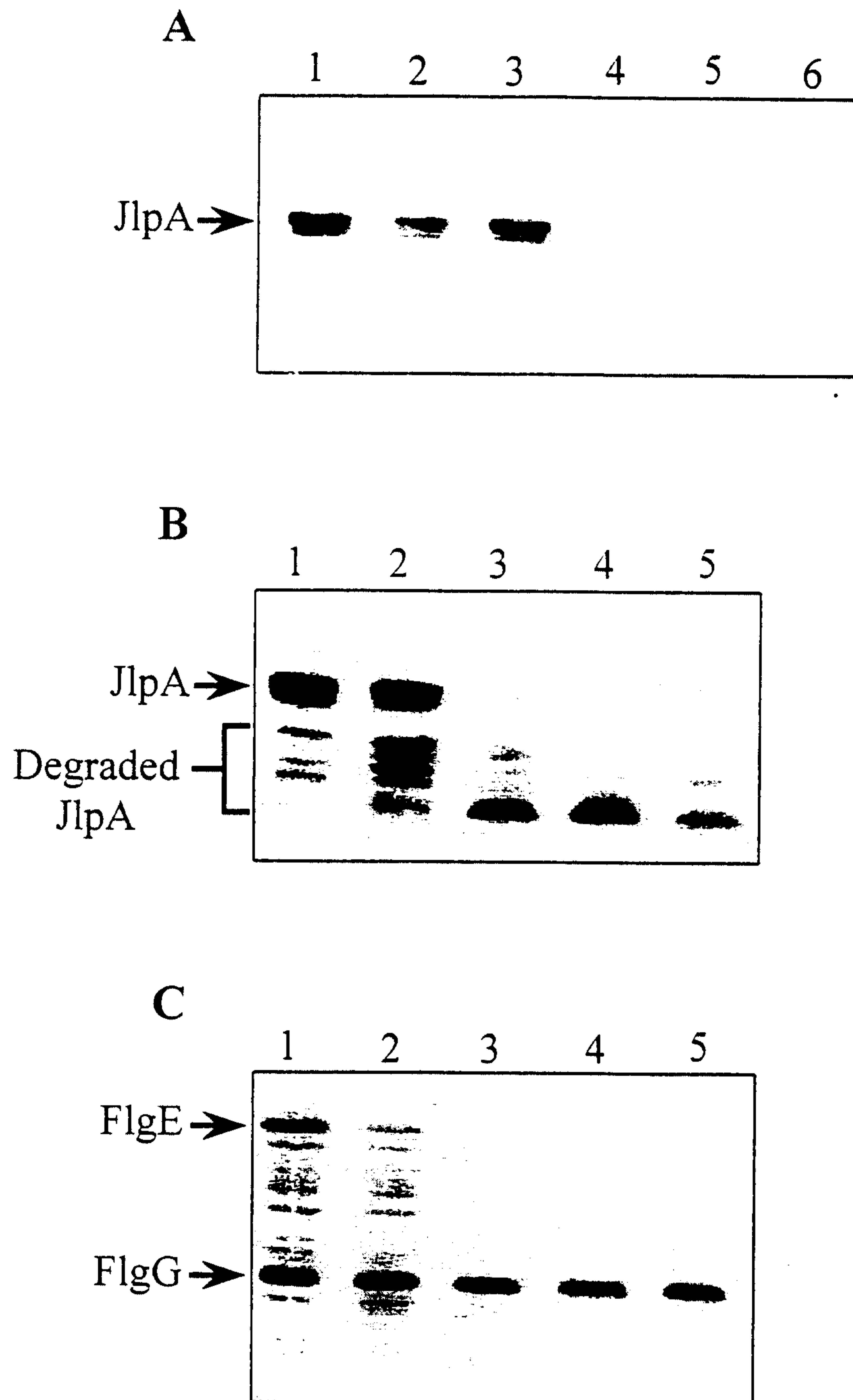


FIGURE 4

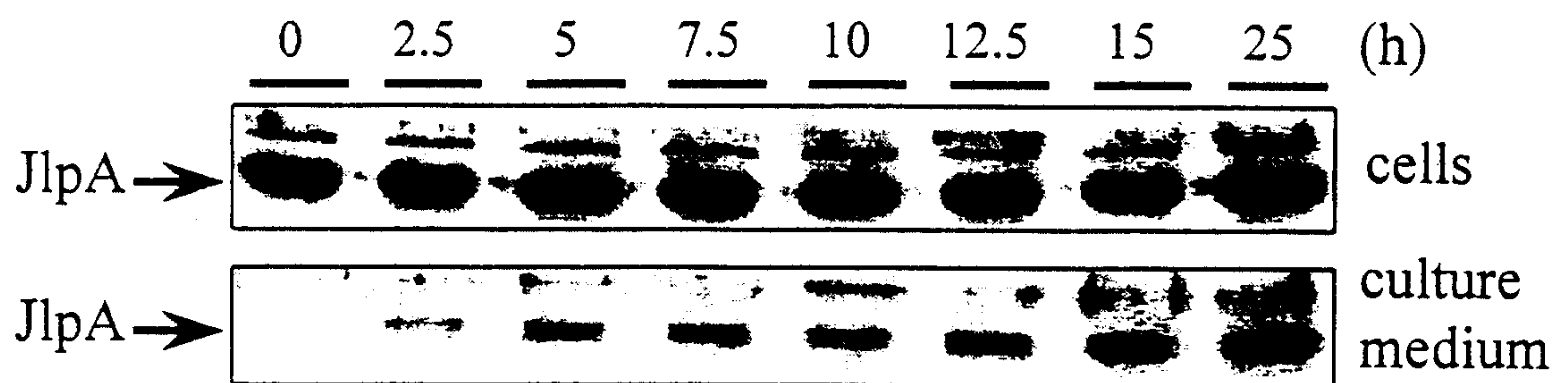
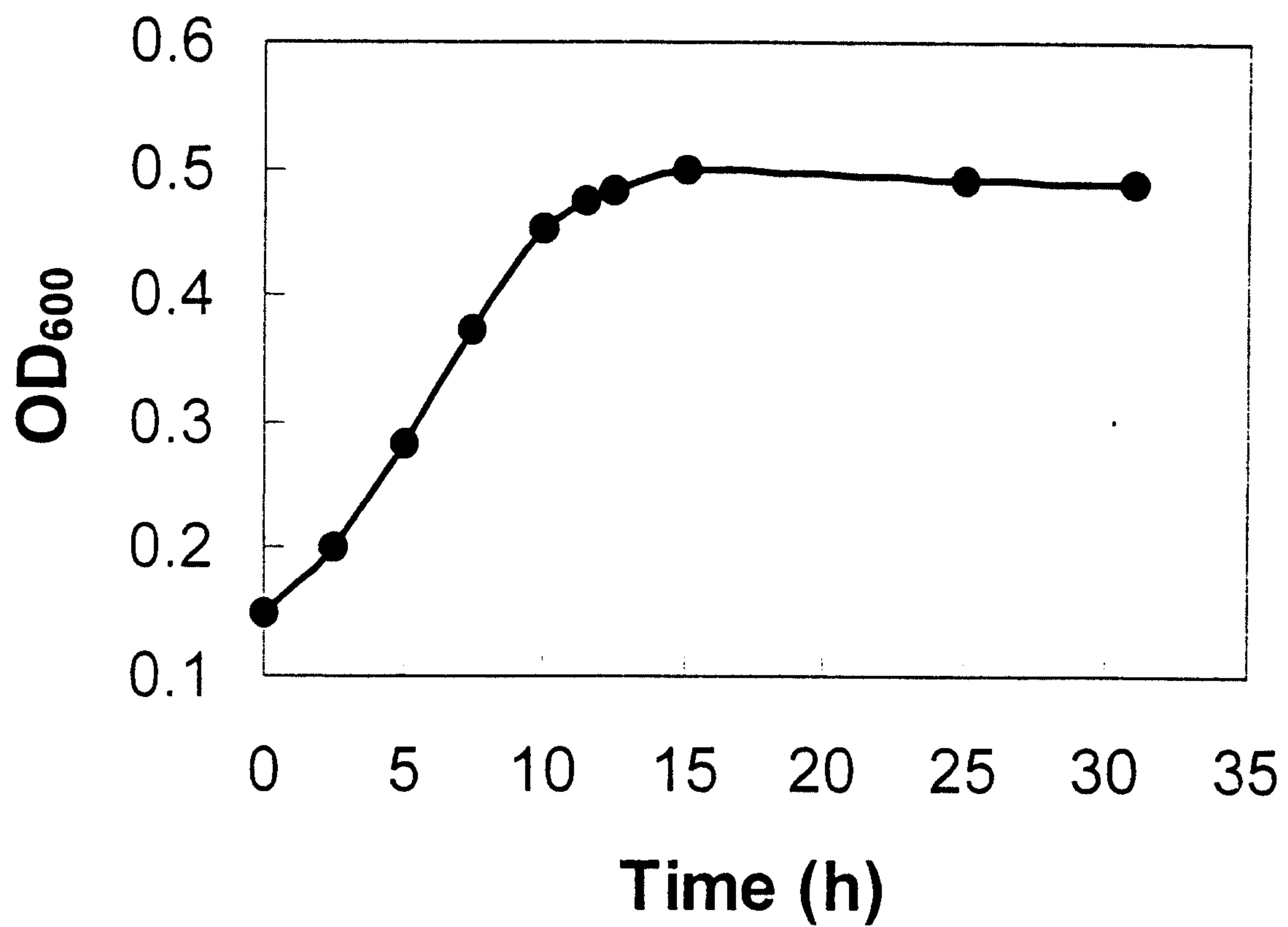


FIGURE 5

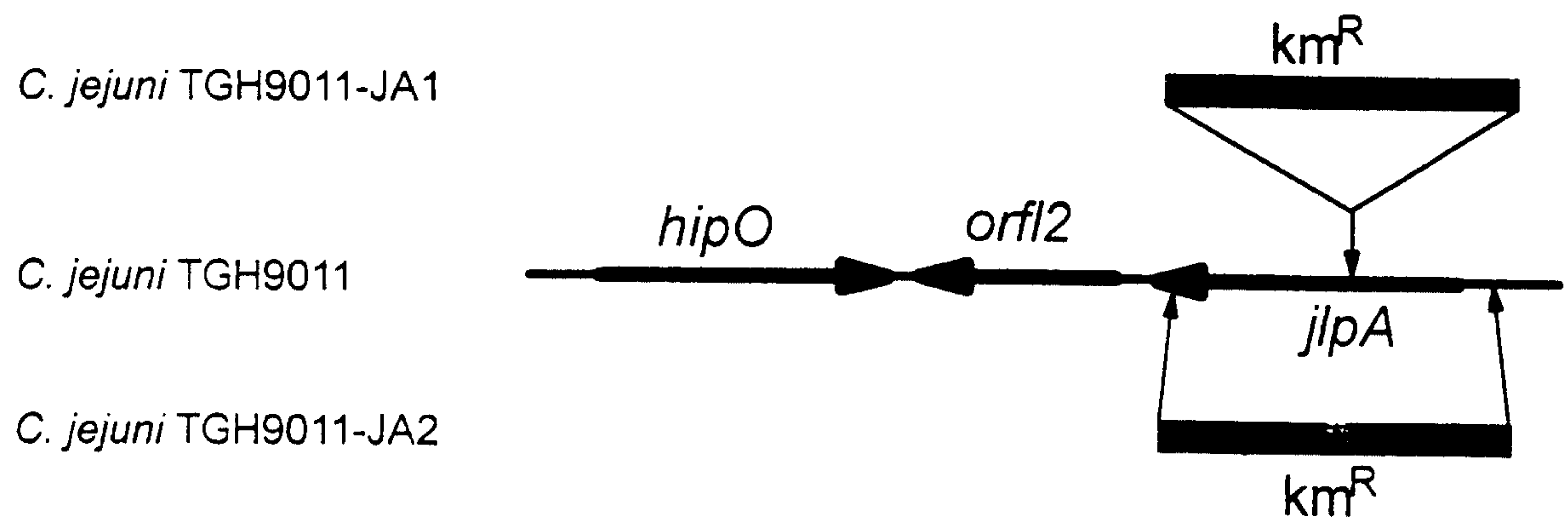


FIGURE 6

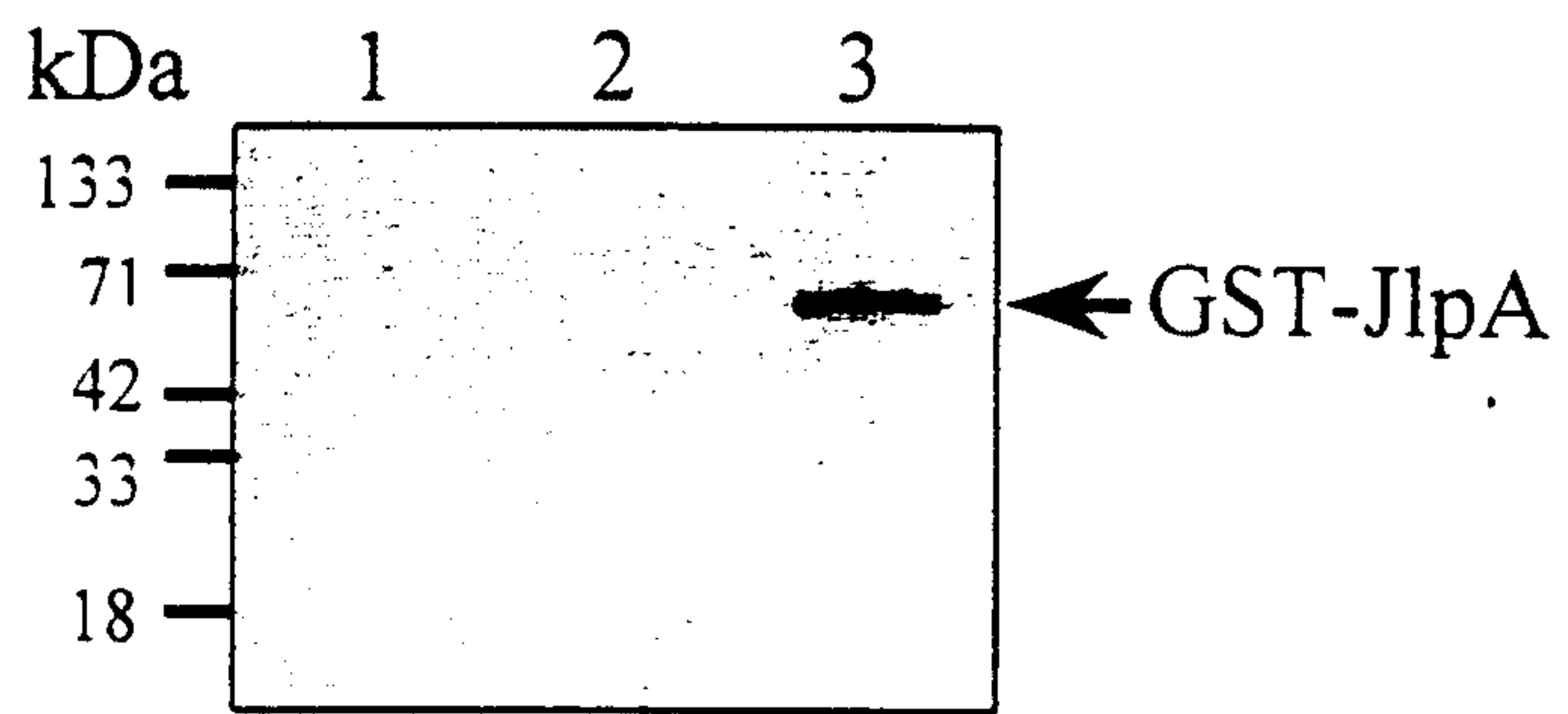


FIGURE 7

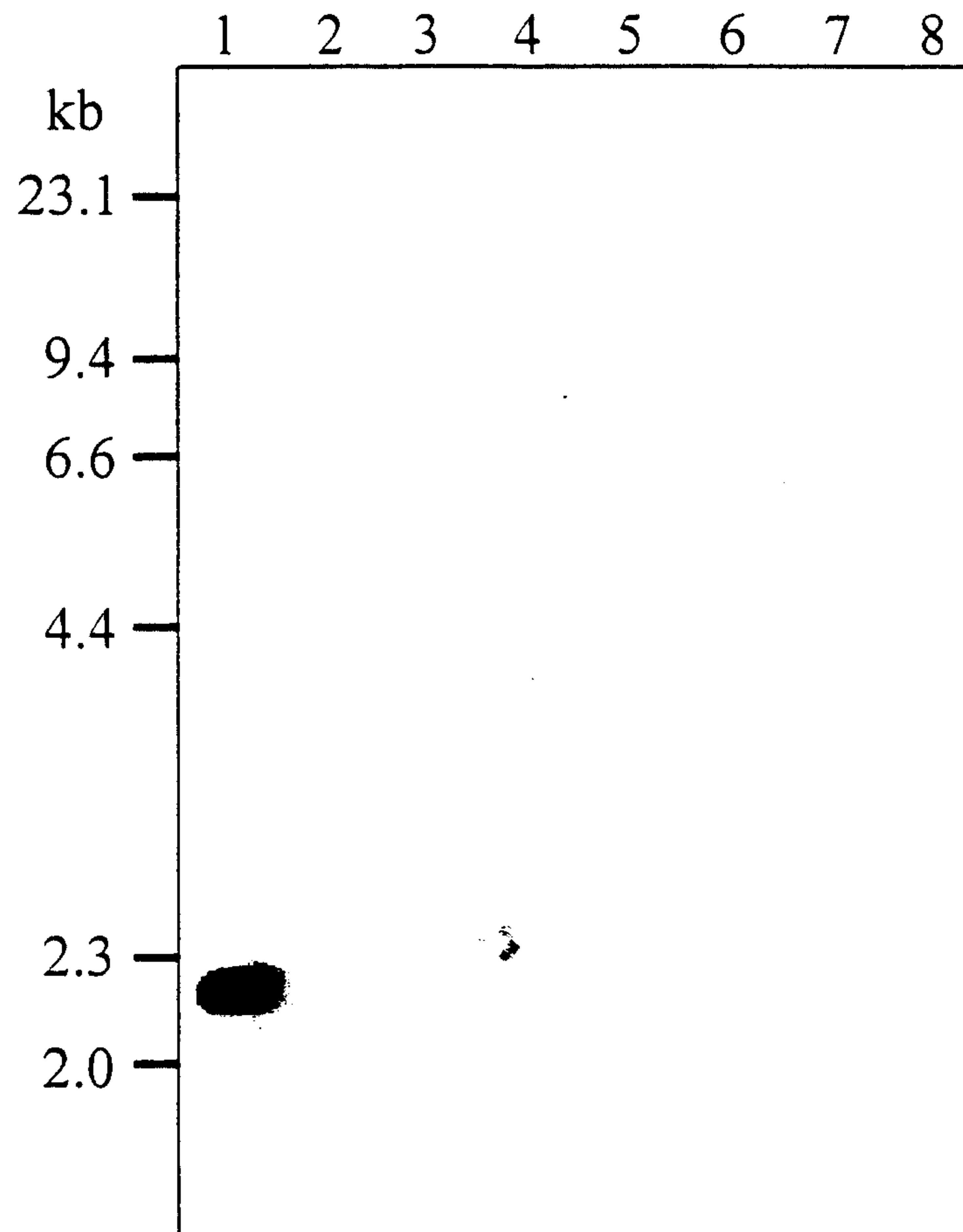
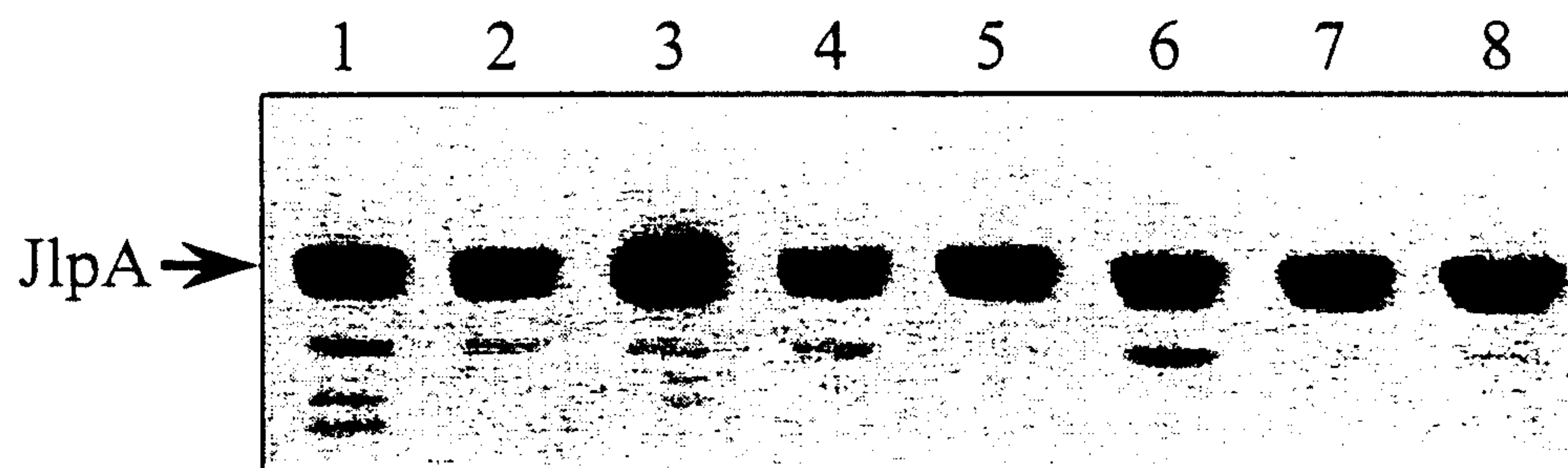
A**B**

FIGURE 8