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(57) Abrégé/Abstract:
Nucleic acids which preferably hybridize under the hybridization conditions 5 - 6 x SSC and 42°C to 60°C with a 2.5kb large KpnI-BamHI fragment from pLM63, preferably with a nucleic acid according to Table III. Such nucleic acids are suitable for hybridization tests for Listeria bacteria if they contain reporter groups. Proteins are also described which are suitable for the production of antibodies against Listeria bacteria.

A b s t r a c t

Method for the determination of pathogenic Listeria bacteria.

Nucleic acids which preferably hybridize under the hybridization conditions 5 - 6 x SSC and 42°C to 60°C with a 2.5kb large KpnI-BamHI fragment from pLM63, preferably with a nucleic acid according to Table III. Such nucleic acids are suitable for hybridization tests for Listeria bacteria if they contain reporter groups. Proteins are also described which are suitable for the production of antibodies against Listeria bacteria.

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Method for the determination of pathogenic Listeria bacteria

The invention concerns a method for the determination of pathogenic Listeria bacteria, a nucleic acid probe, as well as a protein which is suitable for the production of antibodies against pathogenic Listeria bacteria.

Listeria are a heterogeneous group of gram-positive bacteria and consist essentially of the species Listeria monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii, L. grayi and L. murrayi. Merely two of these species are pathogenic, namely L. monocytogenes for humans and animals and L. ivanovii for animals. Listeriosis usually manifests itself in humans as a bacterial meningitis and septicaemia as well as miscarriages and stillbirths in pregnant women. In sheep and cattle listeriosis manifests itself as miscarriage, encephalitis, septicaemia and mastitis. (N. Engl. J. Med. 308 (1983), 203-206, J. Infec. 15 (1987), 165-168, Linnan, M.J. et al., An investigation of listeriosis in Southern California 1985, in Courtieu, A.L. et al., (eds) Listeriose, Listeria, Listeriosis 1985-1986, University of Nantes).

Recently an increasing number of listeriosis diseases have been observed in humans, the cause of which is regarded to be due to the contamination of milk and cheese, above all soft cheese varieties, by Listeria bacteria. Therefore an examination of food for Listeria contamination is important and is obligatory for cheese in the USA.

A method for the determination of Listeria monocytogenes is known from Int. J. Food Microbiol. 4 (1987), 249-256 in which the microorganism is enriched selectively in a liquid medium at 4°C, cultured on special agar plates and subsequently the biotype and serotype determined by a multitude of biochemical tests. This method is very laborious and time-consuming and it takes 14 days and longer before the end result is available. In addition the assessment is very difficult to carry out and is not suitable for routine investigation.

A method for the determination of Listeria bacteria is known from Appl. Environ. Microbiol. 53 (1987), 2256-2259 which is carried out by colony-hybridization with radioactively labelled DNA probes. Colony-hybridization in itself would be a suitable method for a rapid and simple analysis of the contamination of food by Listeria bacteria. However, a nucleic acid probe is necessary for this which hybridizes with all pathogenic Listeria bacteria (L. monocytogenes and L. ivanovii) but not with the other Listeria bacteria. DNA probes known up to now (listeriolysin 0, β-listeriolysin; cf. Infection and Immunity 55 (1987), 3225-3227 and Applied Environmental, Microbiology 53 (1987), 2256-2259) are too non-specific and therefore result in an inacceptable number of false positive and false negative results.

Brief description of the drawings:

Figure 1 (Figs. 1A-D) shows the DNA sequences of dth 18 (nucleotide 822 - 1890) and of a preferred section of the 2.5 kb large KpnI-BamHI fragment of plasmid pLM63 (nucleotide 1 - 1890) as well as the amino acid sequences of proteins I, II and III including the start codon.

The object of the present invention is therefore to provide a nucleic acid with which specific hybridization tests for pathogenic Listeria bacteria are possible as well as proteins derived from this which are suitable as immunogens for the production of antibodies against pathogenic Listeria bacteria.

This object is achieved by a nucleic acid which hybridizes, preferably within an hour, with the 2.5 kb large KpnI-BamHI fragment from the plasmid pLM63 (DSM 5220) under the hybridizing conditions 5 - 6 x SSC and 42 - 60°C. The nucleic acid is preferably at least 27 nucleotides long. A nucleic acid is likewise preferred which hybridizes under these conditions with the nucleic acid sequence according to Figure 1, contained in the above mentioned fragment. A nucleic acid is likewise preferred which hybridizes under these conditions with the nucleic acid corresponding to nucleotide 822 - 1890 of Figure 1. It is particularly preferable to use the nucleic acid with the sequence according to Figure 1, a fragment of this sequence from nucleotide 1 - 714, 822 - 1890, 935 - 1244, from 1158 - 1185 or from 1209 - 1718 or a fragment of the sequence according to Table III of at least 27 nucleotides length. The maximum length of the probe is not critical. Usually it should not considerably exceed the length of the pLM63 fragment i.e. 2.5kb.

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In the sense of the invention nucleic acids are understood as oligodeoxyribonucleosides or the corresponding oligoribonucleosides as well as their derivatives which are suitable for hybridization.

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The Listeria test is carried out by molecular biological techniques familiar to the expert of DNA/DNA, RNA/RNA or RNA/DNA hybridization for the detection of homologous nucleic acid sequences. For this the methods of colony or plaque hybridization and blotting procedures (e.g. Southern blot and dot blot) are usually used. Further known procedures are described in USP 4 358 535; Gene 21 (1983) 7785; publication number EP 130515-A3 of Dattagupta et al., published October 5, 1988; publication number EP 70685-B1 of Heller et al., published October 16, 1985; publication number EP 70687-B1 of Heller et al., published October 16, 1985; and publication number EP 238332-A2 of Goodson et al., published September 23, 1987.

The labelling of the probe is carried out for example with radioactive derivatized deoxyribonucleoside triphosphates, non-radioactively with biotin, avidin, streptavidin, a fluorescent dye or a hapten. In the latter case the detection of the product of hybridization is carrie out by the determination of the enzymatic activity of the marker enzyme in an anti-hapten-antibody-enzyme conjugate via coupled dye systems. Further procedures are for example described in publication number DE 3813278-A1 of Hoeltke et al., published July 20, 1989 and publication number DE 3800644-A1 of Hoeltke et al., published July 20, 1989. The substance with which the probe is labelled is also denoted there as reporter group.

The method in accordance with the present invention is preferably carried out by labelling the nucleic acid according to the present invention radioactively, or with biotin, streptavidin, avidin or with a hapten and subsequently hybridizing and determining the label.

Various methods can be applied to label the probes according to the present invention as described for example in Molecular Cloning, Maniatis et al. (1982), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. The incorporation of biotin in nucleic acids is described for example in PNAS USA 78 (1981) 6633 and PNAS USA 79 (1982) 7331. A further method for the incorporation of reporter groups is described in publication number EP 292128-A1 of Segev, published November 23, 1988.

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The radioactive labelling can be carried out according to the known procedures. The incorporation of a hapten can be carried out for example enzymatically, chemically or photochemically. The preparation of the oligonucleotide probes can for example be carried out according to the "random-primed" method (Anal. Biochem. 132 (1983) 6) the "specific-primed" method, the "reverse transcription" method (Stelow, J.K. and Holländer A. Eds, Genetic Engineering, Plenum Press New York and London, Vol. 1, page 1), according to the "fill in" the "nick-translation"method (J. Mol. Biol. 113 (1977) 237) the "tailing"method, the "transcription" method (J. Mol. Biol. 166 (1983) 477), the photochemical method (Nucl. Acids Res. 13 (1985) 745 - 761), or be carried out chemically. These methods are for example described in more detail in DE-A 38 13 278.

The material to be examined (sample) is prepared in the usual way before the determination is carried out. In this process it is preferable to isolate the RNA and/or DNA from the sample according to known procedures (cf. e.g. Maniatis, Molecular Cloning (1982) 280 - 281).

Before carrying out the determination, the nucleic acids of the sample can be cleaved into shorter chain fragments. This can be effected for example by ultrasonic treatment, treatment with microwaves or treatment with restriction endonucleases. In case the samples contain double-stranded nucleic acids, these must be separated into single strands before the determination is carried out. This can be carried out by

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methods familiar to the expert by denaturation (e.g. heat treatment or treatment with alkali).

The derivatized or radioactively labelled nucleic acid probe is brought into contact with a denatured DNA or RNA of the sample to be examined which is bound to a carrier and in doing this temperature, ionic strength, pH value, buffer, and other conditions are so chosen that, depending on the length of the nucleic acid probe and the resulting melting temperature of the expected hybrid, the labelled nucleic acid can bind to homologous nucleic acids (J. Mol. Biol. 98 (1975), 503, Proc. Natl. Acad. Sci. USA 76 (1979) 368 3). Particularly suitable hybridization conditions are 5-6 x SSC at 42°C - 60°C and an incubation period of 1 to 20 hours, preferably 1 hour. Particularly suitable washing conditions are 0.1 - 0.5 x SSC at 65°C - 70°C and an incubation period of 15 minutes to 2 hours, preferably 30 minutes. To carry out the determination it is however also possible to choose other conditions since the optimal conditions are dependent on the type and concentration of the probe and on the nucleic acid to be determined. Thus for example a lower hybridization temperature can be used by addition of 40 % formamide. Likewise an organic solvent at a concentration of up to 50 %, preferably 20 to 50 %, can be added. Suitable hybridization conditions may be calculated, as described in Anal. Biochem. 178 (1984) 267, from the length of the probe, salt content of the reagent, GC content of the probe and temperature. The following conditions have proven to be particularly suitable:

GC content of the probe 43 % - 60 %,
length of the probe at least 27 nucleotides,
hybridization at 42°C - 60°C and

at 5 - 6 x SSC for 1 hour,
wash at 65°C - 70°C and
at 0.1 - 0.5 x SSC for 30 minutes,
by addition, if desired, of 40 % to 50 % denaturing agent (e.g. formamide) during the hybridization.

1 x SSC is understood as an aqueous solution of

0.15 mol/l NaCl
0.015 mol/l Na_3 citrate x 2 H_2O,

which has been adjusted to pH 7 with 1 mol/l HCl. Details such as 0.1 x SSC are corresponding dilutions of this solution.

Membranes or carrier materials based on nitrocellulose are suitable as carriers (e.g. Schleicher and Schüll BA85, Amersham Hybond C*), reinforced or bound nitrocellulose in powder form or nylon membranes derivatized with various functional groups (e.g. nitro group) (e.g. Schleicher and Schüll Nytran*, NEN Gene Screen*, Amersham Hybond N*, Pall Biodyne). Before carrying out the determination the carrier is preferably pretreated in order to prevent non-specific binding of the probes to the carrier. Blocking substances suitable for the pre-hybridization are for example phosphate buffered saline with bovine serum albumin, non-ionic detergents, polyanions and DNA from herring sperm. Nitrocellulose filters and nylon membranes are preferably pretreated with 5 x SSC at 65°C for 1 hour.

After carrying out the incubation the carrier is washed in order to remove unhybridized nucleic acids. This can

* trade-marks

be effected for example by a solution of 0.1 - 1 % (v/v) of an ionic detergent with addition, if desired, of a salt (e.g. sodium chloride or sodium citrate) or with SSC preferably 0.1 - 0.5 x SSC.

When using radioactively labelled probes an auto-radiography is carried out.

When using probes labelled with hapten, incubation is with an antibody or antibody fragment against the hapten. The antibody thereby carries a label for example a radioactive or enzyme label. After the incubation with the antibody it is washed again in order to detect only those antibody conjugates which are bound specifically. The determination is then carried out via the label of the antibody or the antibody fragment according to well-known methods. An analogous procedure applies for a biotin or avidin label.

The labelling of the anti-hapten antibody or the antibody fragment is carried out according to well-known methods. Suitable are e.g. an enzyme label, radioactive label, (bio)luminescent label or fluorescent label. An enzyme label is however preferably used with enzymes such as alkaline phosphatase, peroxidase or β-galactosidase. Particularly preferred as the enzyme label is alkaline phosphatase. The determination of alkaline phosphatase is carried out via leuco systems, especially via indigoid systems as oxidizable compounds (publication number EP 228663-A3, of Steaffens, published June 1, 1988). Tetrazolium salts serve as oxidizing agents. For the enzyme label alkaline phosphatase X-phosphate/nitroblue-tetrazolium is preferably used as the redox system (F.P. Altmann, Tetrazolium salts and Formazans, Progr. Histochem. Cytochem. Vol. 913 (1976), Gustav-Fischer-Verlag Stuttgart, page 1). In this

connection, X-phosphate is understood as 5-bromo-4-chloro-3-indolylphosphate and nitroblue-tetrazolium as 3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-bis-5-phenyl-2-(4-nitrophenyl)-tetrazolium chloride. Alkaline phosphatase cleaves the chromogenic substrate (X-phosphate) which forms a blue, sparingly soluble dimer by cleavage of the phosphate and oxidation which simultaneously reduces the tetrazolium compound to a likewise blue, sparingly soluble formazan.

The detection of the other suitable labelling systems is carried out according to well-known methods.

A further embodiment of the invention is a reagent for the determination of listeriosis which is characterized in that it has a labelled nucleic acid which has a length of at least 27 nucleotides and which hybridizes at 5 - 6 x SSC and 42°C - 60°C with the 2.5 kb large KpnI-BamHI fragment from the plasmid pLM63 (DSM 5220). A reagent is preferred which contains a labelled nucleic acid which hybridizes with a nucleic acid according to Figure 1 under the conditions mentioned above. Also preferred is a reagent which contains a labelled nucleic acid which hybridizes with a nucleic acid corresponding to the nucleotides 822 - 1890 of Figure 1 under the above-mentioned conditions. The hybridization period is preferably 1 - 20 hours, particularly preferred is 1 hour. Furthermore the reagent contains a detection system for the label. The above mentioned procedures can be used for the labelling.

A labelled nucleic acid corresponding to the sequence of Figure 1 or corresponding to nucleotides 1 - 714, 822 - 1890, 935 - 1244, 1158 - 1185 or 1209 to 1718 is preferably used.

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A further embodiment of the invention is a protein which is at least 80 % homologous to the amino acid sequence of protein I, protein II or protein III cited in Figure 1. A protein is preferred which is at least 80 % homologous to protein II. This protein has a molecular weight of 18 kD. The proteins are suitable for the production of antibodies against Listeria. The proteins with the amino acid sequence according to Figure 1 or the partial sequences protein I, protein II or protein III are preferred. Particularly preferred is protein II.

With the protein according to the present invention, which is modified if desired, antibodies and antisera against pathogenic Listeria bacteria can be obtained by immunizing experimental animals, collecting antiserum and purifying the antibodies according to well-known procedures. Monoclonal antibodies can be obtained by immunizing experimental animals with protein in accordance with the present invention which is modified if desired, fusing B-lymphocytes of the immunized animals thus obtained with transforming agents, cloning and culture of the hybrid cells thus formed which produce the monoclonal antibody and isolation of the latter. Rats and mice are particularly suitable animals for the production of antibodies. Suitable modifying agents are for example N-bromo-succinimide (NBS) by oxidation of tryptophan groups on the protein (BBA 143 (1967) 462-472) carboxymethylation with iodoacetate (IAA) which mainly attacks histidine or nitration with tetranitromethane (TNM) (J. Biol. Chem. 238 (1963) 3307) as well as diazotization with diazotized sulphanilic acid (Meth. Enzymol. 25 (1972) 515-531). Balb/c mice or AJ mice are particularly suitable as the experimental animals.

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The immunization is carried out by the usual administration of the native or modified enzyme preferably in combination with an adjuvant. Freund's adjuvant or aluminium hydroxide together with Bordetella pertussis are preferably used as the adjuvant. The immunization is usually carried out for at least 2 and preferably for 4 months.

In order to isolate monoclonal antibodies the B-lymphocytes of the immunized animals are fused by transforming agents according to the usual methods. Examples of transforming agents used within the scope of the present invention, are myeloma cells, transforming viruses such as e.g. Epstein-Barr virus or agents which are described in publication number DE 3245665-A1 of Jungfer et al., published November 10, 1983. The fusion is carried out according to the well-known procedure of Köhler and Milstein (Nature 256 (1975) 495-497). The hybrid cells which are formed in this process are cloned in the usual manner e.g. by use of a commercial cell sorter and the clones obtained which form the desired monoclonal anti-bodies are cultured. The production and selection of monoclonal antibodies is for example described in detail in J. Immunol. Meth., 39 (1980) 285-308.

A further embodiment of the invention is an immunological method for the determination of Listeria bacteria in which at least one monoclonal or, if desired, polyclonal antibody is used against the protein according to the present invention. In principle all current immunoassays such as e.g. radioimmunoassay, enzyme-immunoassay, fluorescence-immunoassay etc. are suitable as immunological determination methods. Furthermore all variants of the procedures such as e.g. the competitive immunoassay IEMA method can be used. The usual agents for the respective determination method are

suitable for the labelling. Thus radio-isotopes, for example ^{125}I are used for the labelling in a radioimmunoassay. For an enzyme-immunoassay all the enzymes which are usually used, such as for example peroxidase or β-galactosidase, are suitable. For a fluorescence immunoassay the usual fluorescent groups can be used as the label. Details of these various test methods and variants of the procedures are known to the expert.

In the determination method the monoclonal or polyclonal antibodies as such or fragments thereof can be used which have the corresponding immunological properties for example Fab fragments.

The following Examples and the Figures elucidate the invention further. The quoted percentages are percentages by weight unless stated otherwise.

Example 1

Labelling of the DNA probes

200 ng of the isolated DNA are labelled with ^{32}P as described in Anal. Biochem. 132 (1983), 6-13.

Example 2

Preparation of the samples

2.1 Microorganisms

The strains used as a reference are listed in Tab. Ia. All the other Listeria strains (Tables Ib, II) were chosen from a collection of about 3000 Listeria strains of the National Institute of Public Health and Environmental Protection, Bildhoven. Among the strains examined are 40 strains which originate from patients who suffer from listeriosis. These strains were isolated as described in Zbl. Bact. I Abt. Orig. A 246 (1980), 211 - 227. The microorganisms were cultured for 24 hours at 30°C in a liquid selection and enrichment medium (pH 7.0, 1 g/l peptone, 8.5 g/l NaCl, 10 µg/ml trypaflavin·HCl, 10 µg/ml nalidixic acid, 50 µg/ml cycloheximide) and subsequently plated on a special nutrient medium (enrichment medium + 1 % agar). After incubation at 30°C for 24 hours these plates are used directly in the hybridization test.

2.2 Other sample material

Cerebrospinal fluid, faeces, blood, lochia, brain material, liver, cervical material, amniotic fluid, food, silage grass and animal material are used as additional sample material. 20 g of this material is suspended in 250 ml phosphate buffered saline, homogenized and further processed as described in International Journal of Food Microbiology 4 (1987), 249-256. 0.1 ml sample is plated on non-

selective agar plates (85 mm in diameter) and incubated overnight.

2.3 Procedure for a hybridization test with a DNA probe (dth 18, 1069 nucleotides or pLM63, 1890 nucleotides, Figure 1)

A replica of the plates prepared according to 2.1 or 2.2 was made onto nylon membranes (Gene Screen Plus Membranes^{R}, DuPont Corp., USP 4,455,370). The membranes are placed on filter paper which is saturated with 0.5 mol/l NaOH and incubated for 5 minutes in a boiling water-bath. Afterwards the membranes are placed on filter paper which is saturated with 1 ml 0.5 mol/l of fresh NaOH and neutralized with 1 ml 1 mol/l Tris buffer (pH 7.5). This neutralization step is repeated. The membranes are dipped in 100 ml 5 x SSC and at the same time the cell residues are rubbed off with a cloth. After drying in air the membranes are pre-hybridized with 15 ml solution A for 6 to 16 hours at 40°C. Afterwards the DNA probe dth 18 or pLM63 (0.05 μg in 2.5 ml, 5 x SSC) is added and incubated for 18 hours at 60°C. Afterwards they are washed for 45 min in a water-bath at 65°C with 0.2 x SSC which contains in addition 1 % SDS and 0.1 % sodium pyrophosphate. After drying, the membranes are shrink-wrapped in plastic foil and an X-ray film is exposed with them for 18 hours at -70°C.

2.4 Hybridization with a 314bp DNA probe

Hybridization tests are carried out with a shortened probe (nucleotide 1209 - 1718 (cf. Figure 1). The conditions for the hybridization are 5 x

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SSC at 42°C (1 hour) and subsequent washing in 0.2 x SSC at 70°C (30 minutes). It can be seen that this probe is also suitable as a DNA probe to test for listeriosis.

Reagents:

1 x SSC: 0.15 mol/l NaCl and 0.015 mol/l sodium citrate

Solution A:

50 mmol/l Tris buffer, pH 7.5
10 mmol/l EDTA
1 mol/l NaCl
0.2 % Ficoll*
0.2 % polyvinylpyrrolidone
0.2 % bovine serum albumin
1 mg/ml denatured DNA from herring sperm
1 % sodium pyrosphate and
10 % SDS (sodium dodecyl sulphate).

For the further examinations less stringent conditions (2 x SSC at 65°C) were used. Analogous results were thus obtained.

E x a m p l e 3

Comparative experiments with a DNA probe according to the state of the art (listeriolysin 0)

A synthetic 19 mer-oligomer (^{5}GATCACTCTGGAGGATACG^{3}) was used as the probe (analogous to Infection and Immunity 56 (1988), 766 - 772).

* trade-mark

200 ng of this DNA probe was labelled at the 5'-end with ^{32}P as described in Maxam and Gilbert (Meth. Enzymol. 65 (1980), 499).

The hybridization conditions were:

6 x SSC at 37°C, washing three times with 6 x SSC containing 1 % SDS and 0.1 % sodium pyrophosphate at 40°C for 30 min each.

E x a m p l e 4

Determination of the biotype of the microorganisms used

The determination of the biotype was carried out as described in An. Inv. Pasteur/Microbiol. 134 (1983) 56 - 71. The strains were cultured for two days at 37°C in 4 ml semisolid peptone-agar medium which contained 1 % D-xylose or L-ramnose. The peptone medium consists of 10 g bacto-peptone, 5 g sodium chloride, 5.5 oxoid-agar L 28 and 0.08 g bromthymol blue dissolved in 1000 ml distilled water, pH 7.8 autoclaved at 120°C for 15 min. After the autoclaving, filter-sterilized sugar solution was added up to a final concentration of 1 %. In order to determine the biotype haemolysin production was examined by growing the strains overnight at 37°C in brain heart infusion broth. 0.2 ml are withdrawn from this culture and added to 0.2 ml of a 2 % suspension of sheep erythrocytes in PBS (phosphate buffered saline) which was washed three times. After incubation for two hours at room temperature it is tested for haemolysis. L. monocytogenes, NCTC7973 and L. innocua, NCTC11289 served as the positive and negative controls.
The determination of the biotype was carried out

according to Zbl. Bakt. Mikrobiol. Hyg. I Abt. Orig. A 259 (1985) 341 - 350.

E x a m p l e 5

Determination of the serotype

The determination of the serotype was carried out as described in Seeliger and Höhne (Zbl. Bact. Microbiol. Hyg. I Abt. Orig. A, 259 (1985), 341-350).

The strains were cultured in 4 ml tryptone phosphate broth, enriched with 1 % glucose and shaken for 6 - 7 hours at 37°C in a water-bath. A tryptose-agar plate is inoculated with this culture and incubated overnight at 37°C. Paralell to this a second tube containing tryptose phosphate broth to which 1 % glucose was added, is inoculated and incubated and shaken overnight at 25°C in a water-bath. The tryptose phosphate plate is harvested with 5 ml phosphate buffered saline, pH 7.4 and heated for one hour in a boiling water-bath and subsequently centrifuged.

The cells are resuspended and adjusted to a concentration of 5×10^8 cells/ml (1×10^9 cells/ml have an OD of 1.0 at 600 nm). 0.2 ml are withdrawn from this suspension and added to 0.2 ml of a 75-fold dilution of antiserum against the polysaccharide antigens I, II, V, VI, VIII and IX in a glass tube and incubated for 2 hours at 45°C. Afterwards it is cooled and incubated overnight at 4°C. Afterwards the tubes are tested for visible agglutination.

The determination of the serotype is carried out as described in detail in Bergeys Manual of Bacteriology (N.R. Kreig, J.G.Holt (eds) Bergeys Manual of Systematic Bacteriology (1984), Williams and Wilkins, Baltimore and London). The serotype was classified into the classes 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4d, 4a/b, 5, 6b and 7.

Example 6

Mouse bioassay

A mouse bioassay (as described in Infect. Immun. 45 (1984), 234 - 241) is carried out in order to examine whether a Listeriosis strain is pathogenic. For this the mouse is injected intravenously with 10^4 - 10^5 bacteria in 200 µl phosphate buffered saline pH 7.4. After 2 - 4 days the mice are killed and the spleen is removed. A strain is denoted pathogenic when the number of bacteria found in the whole spleen exceeds 10^4.

Example 7

Preparation of Listeria antibodies

50 µg of the purified antigen (amino acid sequence Figure 1) in complete Freund's adjuvant is injected intramuscularly (IM). The injection is repeated after three weeks, after 30 days and after 40 days. 10 days after the last injection antiserum is isolated and purified by affinity chromatography.

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An immunoblot can be carried out with this antiserum for the determination of Listeria.

Example 8

Examination of the suitability of a probe for the determination of Listeria

E. Coli HB 101 (DSM 1607) are transformed with a plasmid based on pUC18 which contains as an insert the DNA sequence according to Figure I and cultured overnight in the presence of 50 µg/ml ampicillin up to an optical density of OD 600 nm = 1. 0.1 ml of this culture broth is plated on agar plates which contain 50 µg/ml ampicillin (85 mm in diameter) and incubated overnight.

A replica is made of the plates thus produced using nylon membranes (Gene Screen Plus-Membranes^{R}, DuPont).

The membranes are placed on filter paper which is saturated with 0.5 mol/l NaOH and incubated for 5 minutes in a boiling water-bath. Afterwards the membranes are placed on filter paper which is saturated with 1 ml 0.5 mol/l of fresh NaOH and neutralized with 1 ml 1 mol/l Tris buffer (pH 7.5). This neutralization step is repeated. The membranes are dipped in 100 ml 5 x SSC and at the same time the cell debris are rubbed off with a cloth. After drying in air the membranes are pre-hybridized with 15 ml solution A for 6 to 16 hours at 40°C. Afterwards the DNA probe which is to be examined for its suitability (0.05 µg in 2.5 ml) is added and incubated for 18 hours at 60°C. Afterwards they are washed for 45 min in a water-bath at 65°C with 0.2 x SSC which contains in addition 1 % SDS and 0.1 %

sodium pyrophosphate. After drying, the membranes are shrink-wrapped in plastic foil and an X-ray film is exposed with them for 18 hours at -70°C. If signals are visible on the X-ray film which originate from a hybridization, this probe is suitable for use in the listeriosis test.
When plasmid pLM63 (DSM 5220) is used analogous results are obtained.

Example 9

Comparison of the hybridization of a probe according to the present invention and a reference probe (cf. Example 3) with various Listeriosis strains. The hybridization was carried out as described in Examples 2 and 3.
The results are shown in Table I and II.

Table I

Comparison of the hybridization of a DNA probe according to the present invention (1) (935bp to 1244 bp of Figure 1) and a DNA probe according to the state of the art (listeriolysin 0, (2), prepared according to Example 3) with DNA from Listeria strains.

Analogous results are obtained with nucleotide 1 - 714 as DNA probe (1). This probe hybridizes in addition with DNA from Listeria monocytogenes strains of the serotype 4a (e.g. NCTC 5214).

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Table Ia:

with reference strains

Sero-type	Strain No.[1)]	Biotype	probe 1	probe 2
1/2 a	NCTC7973	L.monocytogenes	+	+
1/2 b	SLCC2755	L.monocytogenes	+	+
	SLCC3954	L.seeligeri	-	-
1/2 c	NCTC5348	L.monocytogenes	+	+
3 a	NCTC5105	L.monocytogenes	+	+
3 b	SLCC2540	L.monocytogenes	+	+
3 c	SLCC2479	L.monocytogenes	+	+
4 b	NCTC10527	L.monocytogenes	+	+
4 c	ATCC19116	L.monocytogenes	+	+
4 d	NCTC10888	L.monocytogenes	+	+
4 e	ATCC19118	L.monocytogenes	+	+
4 ab	NCTC10528	L.monocytogenes	+	+
5	ATCC19119	L.ivanovii	+	-

Sero-type	Strain No.[1]	Biotype	probe 1	probe 2
6 a	SLCC 5334	L.welshimeri	-	-
	NCTC11288	L.innocua	-	-
6 b	NCTC11289	L.innocua	-	-
7	SLCC 2482	L.monocytogenes	+	+
	RIVM 1	L.gravi	-	+
	RIVM 2	L.murravi	-	+

1) Repositories cf. World Directory of Collections of Cultures of Microorganisms edited by V.F. McGowan and V.B.D. Sherman, World Data Center, University of Queensland, Australia, 1982.

T a b l e Ib:

with a multitude of strains

Number of strains examined	Biotype		probe 1	probe 2
34	L.monocytogenes	(34)	34	34
39	L.monocytogenes	(37)	37	37
	L.seeligeri	(1)	0	0
	L.welshimeri	(1)	0	0
16	L.monocytogenes	(16)	16	16
5	L.monocytogenes	(5)	5	5
5	L.monocytogenes	(3)	3	3
	L.welshimeri	(1)	0	0
	L.seeligeri	(1)	0	0
2	L.monocytogenes	(1)	1	1
	L.innocua	(1)	0	0
77	L.monocytogenes	(77)	76	76

Number of strains examined	Biotype		probe 1	probe 2
5	L.seeligeri	(5)	0	0
2	L.monocytogenes	(2)	2	2
1	L.monocytogenes	(1)	1	1
34	L.innocua	(32)	0	1
	L.welshimeri	(2)	0	0
23	L.innocua	(18)	0	2
	L.welshimeri	(5)	0	0
7	L.monocytogenes	(7)	7	7
2	L.grayi	(2)	0	2
3	L.murrayi	(3)	0	3

T a b l e II

Table II shows the hybridization reaction of probe 1 with various Listeria strains whose pathogenicity was determined according to Example 6.

Biotype	Sero-type	Origin	Hybridi-zation	log No./g spleen 2nd Day	4th Day
L.monocytogenes	1/2 a	reference strain	+	7.4[c)]	8.3 +
L.monocytogenes	1/2 b	soft cheese	+	6.2	7.1 +
L.monocytogenes	1/2 c	food	+	5.4	+ +
L.monocytogenes	3 a	food	+	6.4	6.5
L.monocytogenes	3 b	food	+	8.4	+ +
L.monocytogenes	3 c	food	+	8.2	+ +
L.monocytogenes	4 b	soft cheese	+	7.0	+ +
L.monocytogenes	4 b	soft cheese	+	6.5	+ +
L.monocytogenes	4 b	soft cheese	+	8.3	+ +
L. seeligeri	4 c	food	-	<2.0	<2.0

Biotype	Sero-type	Origin	Hybridi-zation	log No./g spleen 2nd Day	log No./g spleen 4th Day
L.innocua	6 a	soft cheese	-	2.0	3.7
L.innocua	6 b	soft cheese	-	<2.0	<2.0
L.grayi		reference strain	-	<2.0	<2.0
L.murrayi		reference strain	-	<2.0	<2.0

a) 10^4-10^5 organisms were injected into 4 mice.

b) Hybridization is carried out with the dth 18 gene as probe (cf. Example 2.3).

c) Mean of two mice;

+: Mouse died before sampling.

The embodiments of the invention in which an exclusive property of privilege is claimed are defined as follows:

1. A nucleic acid which hybridizes to the 2.5kb large KpnI-BamHI fragment from plasmid pLM63 (DSM 5220) under hybridization conditions of 5 - 6 X SSC and 42 - 60°C and after washing for at least 30 minutes at from 0.1-0.5 X SSC and at a temperature from 65°C-70°C.

2. A nucleic acid as claimed in claim 1, wherein said nucleic acid hybridizes to the nucleic acid fragment:

```
ACGCAAAAACCAGTTTTGAAGTAAGTATTTGTCGTGAATTAAAAGTAAAAATTTAGGGGG
  AlaLysThrSerPheGluValSerIleCysArgGluLeuLysValLysIle---
AAATATTAATGGCATTTGAAGAGAATTTATATTGTGATTATACACCGGGAGCTGCTAAAG
          METAlaPheGluGluAsnLeuTyrCysAspTyrThrProGlyAlaAlaLysAla
          ---------- Protein II
CGGTCGCGGGGAAAGATGTAATTTTAGCAGTTTTTAACGCAGCGGGGGACAAACTATTAG
  ValAlaGlyLysAspValIleLeuAlaValPheAsnAlaAlaGlyAspLysLeuLeuAla
CGGTTGCGGGCCAACAAGGTCTAACTGTAAACCGTTCTAAAGATAGCATTGAAATTACAT
  ValAlaGlyGlnGlnGlyLeuThrValAsnArgSerLysAspSerIleGluIleThrSer
CTAAAGATACAGTTGGCGGATGGAAATCCAAAATTGGCGGTATGAAAGAATGGTCAATTG
  LysAspThrValGlyGlyTrpLysSerLysIleGlyGlyMETLysGluTrpSerIleGlu
AAAATGACGGATTATATGTCGCTGATGCAGAGTCTCACAAAGAATTGGCGAAATATTTCG
  AsnAspGlyLeuTyrValAlaAspAlaGluSerHisLysGluLeuAlaLysTyrPheGlu
AAAGTGATAGCCCGGTTTGTGTGAAAATCATTAATCAAGCATCTAAAAAAGGTCTTTTCG
  SerAspSerProValCysValLysIleIleAsnGlnAlaSerLysLysGlyLeuPheGly
GTGGTTTGGCAATTGTAGCTGACTATAGTTTTGAAGCACCTTTTGACGAAGCGATGACTT
  GlyLeuAlaIleValAlaAspTyrSerPheGluAlaProPheAspGluAlaMETThrTyr
ACTCTGTAAAACTAGACGGAATGGGCGCGCTTGTTGATTTAACGATTACTGAGGGCGGCG
  SerValLysLeuAspGlyMETGlyAlaLeuValAspLeuThrIleThrGluGlyGlyAsp
ACCAAATGCCCGGCGAAACACCTGTAGCACCAGCAGAATAAAATAGAAAGCCACTGAAAT
  GlnMETProGlyGluThrProValAlaProAlaGlu---
AAGTGGCTTTCCCTTAGGAGGAAAATAAATGTTTGAAGTGAATGATACAACTTATATTTT
                                  METPheGluValAsnAspThrThrTyrIleLeu
ACGATTTAATAAACAAAAAGTTAAAACGGTGGAATTAACATCAGGGATTAGTTTAGTTGC
  ArgPheAsnLysGlnLysValLysThrValGluLeuThrSerGlyIleSerLeuValAla
AGCTTTGACTGCGAATAAAGGGATTTTGAG
  AlaLeuThrAlaAsnLysGlyIleLeu   ,
```

under hybridization conditions of 5 - 6 x SSC and 42 - 60°C.

3. A nucleic acid as claimed in claim 1, wherein the GC content of the nucleic acid is 43% to 60%.

4. A nucleic acid as claimed in claim 2, wherein the GC content of the nucleic acid is 43% to 60%.

5. A nucleic acid as claimed in claim 1, 2, 3 or 4, which is identical with at least a 27bp long section of the complete nucleic acid sequence:

C

```
CTTTTCATTTAGATAAAACAAAAGAAGAAATTGGCGCTTTACCTGCTTCGGCGATTGAAT
  PheHisLeuAspLysThrLysGluGluIleGlyAlaLeuProAlaSerAlaIleGluCys
GTCAGTATGAGGCTTTTGTGATTAATGAAGCCAATAATTAAGGAGTGATAAAATGCAGGT
  GlnTyrGluAlaPheValIleAsnGluAlaAsnAsn---
                                        METGlnVal
                                        └► Protein III
TTTAGTTTTACCAGAAAATAAGGATATCAATTATATAAAAACGGTCCAAGAAGTAAAACG
 LeuValLeuProGluAsnLysAspIleAsnTyrIleLysThrValGlnGluValLysArg
ATTTTTTGCGGATTTTGAGCGGTTTCGGATGATTACGGGGTTATCAAAAAAGCCACATTT
 PhePheAlaAspPheGluArgPheArgMETIleThrGlyLeuSerLysLysProHisLeu
ACTTAGAAATGGTTTTCTGGAAGAGCCGCAGTTTGAGCCGGTAGCATTTTCTGCTAGACA
 LeuArgAsnGlyPheLeuGluGluProGlnPheGluProValAlaPheSerAlaArgHis
TAATAAAGAAGTCATTTTGGAAGCGCGATGGTTGGTAGAGAAATATACTGAAATGTTGAA
 AsnLysGluValIleLeuGluAlaArgTrpLeuValGluLysTyrThrGluMETLeuAsn
TCAGATGGATGATTTATATCGAACTATTTTGATGGAATGTTACGTGGAACGAAAACAAGA
 GlnMETAspAspLeuTyrArgThrIleLeuMETGluCysTyrValGluArgLysGlnAsp
TGTGGCGGTAATGATGGATTTACCGTATGAAATTGCCCAGTTTAAACGGATAAAAAAACG
 ValAlaValMETMETAspLeuProTyrGluIleAlaGlnPheLysArgIleLysLysArg
GGCAGTGCTAGAACTTGCAACGCTAATGGGGATTTTAGTAAGGAAATGATGATACTTTCG
 AlaValLeuGluLeuAlaThrLeuMETGlyIleLeuValArgLys---
TGATATTTTGAAACATCATTTTCCTATTAATATAGAAGTAAGCTAATTGTCCAGTAAGCG
GATGACAATAAAAGCTGCATCAGAATGAAGGTGCACCGATTTTCTGATAATACATGATGT
TTTACAAGGAATTTGTTTTTATGATTGGATTTAAATCCGTTGAGATAAACAAATATTCTA
TTTTGGAAAGTAAAGTTCGGAGGAATAAATTATTAAATGTGGTCTTGACCGAACTTTGCT
TTCTGTTTTAAAGGAGTGAACGTTTGGTGAAGAGTTTGAGCTTCATGAGAGTTTTGGAAG
                                        ValLysSerLeuSerPheMETArgValLeuGluAla
                                                  └──► Protein I
CAGTGAGAACAATGCTCCAGGAAAAAGGCGGACTAGATATTTCTATTGTAATGCGTGACC
   ValArgThrMETLeuGlnGluLysGlyGlyLeuAspIleSerIleValMETArgAspGl
AAGTGGAAATGCCTACAACGATGATCGAGATGATTGATCAAGAGGAAGAAGAAAGCCAAA
  ValGluMETProThrThrMETIleGluMETIleAspGlnGluGluGluGluSerGlnThr
CTGCCTGGAAAGAAAAATACCGTTTTGCAATCCATCATTATACAAATGAAACGGACTTAG
  AlaTrpLysGluLysTyrArgPheAlaIleHisHisTyrThrAsnGluThrAspLeuAla
CGGGAGTCGAAAAGATAGATACGCTTATCCAAACAGGATTCACTTTGCCTGAAGGATACA
  GlyValGluLysIleAspThrLeuIleGlnThrGlyPheThrLeuProGluGlyTyrLys
AATTAATCGCTGTTCGACATTACGGAAAACAAAATTTAGTCAAAGAAAATACGTTAATTC
   LeuIleAlaValArgHisTyrGlyLysGlnAsnLeuValLysGluAsnThrLeuIleHis
ACGCAAAAACCAGTTTTGAAGTAAGTATTTGTCGTGAATTAAAAGTAAAAATTTAGGGGG
  AlaLysThrSerPheGluValSerIleCysArgGluLeuLysValLysIle---
AAATATTAATGGCATTTGAAGAGAATTTATATTGTGATTATACACCGGGAGCTGCTAAAG
       METAlaPheGluGluAsnLeuTyrCysAspTyrThrProGlyAlaAlaLysAla
       └──► Protein II
CGGTCGCGGGGAAAGATGTAATTTTAGCAGTTTTTAACGCAGCGGGGGACAAACTATTAG
  ValAlaGlyLysAspValIleLeuAlaValPheAsnAlaAlaGlyAspLysLeuLeuAla
CGGTTGCGGGCCAACAAGGTCTAACTGTAAACCGTTCTAAAGATAGCATTGAAATTACAT
  ValAlaGlyGlnGlnGlyLeuThrValAsnArgSerLysAspSerIleGluIleThrSer
CTAAAGATACAGTTGGCGGATGGAAATCCAAAATTGGCGGTATGAAAGAATGGTCAATTG
  LysAspThrValGlyGlyTrpLysSerLysIleGlyGlyMETLysGluTrpSerIleGlu
AAAATGACGGATTATATGTCGCTGATGCAGAGTCTCACAAAGAATTGGCGAAATATTTCG
  AsnAspGlyLeuTyrValAlaAspAlaGluSerHisLysGluLeuAlaLysTyrPheGlu
AAAGTGATAGCCCGGTTTGTGTGAAAATCATTAATCAAGCATCTAAAAAAGGTCTTTTCG
  SerAspSerProValCysValLysIleIleAsnGlnAlaSerLysLysGlyLeuPheGly
GTGGTTTGGCAATTGTAGCTGACTATAGTTTTGAAGCACCTTTTGACGAAGCGATGACTT
  GlyLeuAlaIleValAlaAspTyrSerPheGluAlaProPheAspGluAlaMETThrTyr
ACTCTGTAAAACTAGACGGAATGGGCGCGCTTGTTGATTTAACGATTACTGAGGGCGGCG
  SerValLysLeuAspGlyMETGlyAlaLeuValAspLeuThrIleThrGluGlyGlyAsp
ACCAAATGCCCGGCGAAACACCTGTAGCACCAGCAGAATAAAATAGAAAGCCACTGAAAT
  GlnMETProGlyGluThrProValAlaProAlaGlu---
```

C

AAGTGGCTTTCCCTTAGGAGGAAAATAAATGTTTGAAGTGAATGATACAACTTATATTTT
METPheGluValAsnAspThrThrTyrIleLeu
ACGATTTAATAAACAAAAAGTTAAAACGGTGGAATTAACATCAGGGATTAGTTTAGTTGC
ArgPheAsnLysGlnLysValLysThrValGluLeuThrSerGlyIleSerLeuValAla
AGCTTTGACTGCGAATAAAGGGATTTTGAG
AlaLeuThrAlaAsnLysGlyIleLeu

6. A nucleic acid as claimed in claim 1, 2, 3 or 4, wherein said nucleic acid carries a label.

7. A nucleic acid as claimed in claim 5, wherein said nucleic acid carries a label.

8. A nucleic acid as claimed in claim 6, wherein said nucleic acid is labelled radioactively, with biotin, avidin, streptavidin, a fluorescent dye or a hapten.

9. A nucleid acid as claimed in claim 7, wherein said nucleic acid is labelled radioactively, with biotin, avidin, streptavidin, a fluorescent dye or a hapten.

10. A nucleic acid molecule useful in identifying pathogenic strains of Listeria which hybridizes to the 2.5kb KpnI-BamHI fragment from plasmid pLM63 (DSM 5220) under hybridization conditions of 5 - 6 X SSC and 42 - 60°C and after washing for at least 30 minutes at from 0.1-0.5 X SSC and at a temperature from 65°C-70°C.

11. A nucleic acid molecule of claim 10, further comprising a label.

12. A nucleic acid molecule of claim 11, wherein said label is radioactive, biotin, avidin, streptavidin, a fluorescent dye or a hapten.

13. A protein which is at least 80% homologous to the amino acid sequence of one of protein I,

protein II, and protein III as set forth in sequence:

```
CTTTTCATTTAGATAAAACAAAAGAAGAAATTGGCGCTTTACCTGCTTCGGCGATTGAAT
  PheHisLeuAspLysThrLysGluGluIleGlyAlaLeuProAlaSerAlaIleGluCys
GTCAGTATGAGGCTTTTGTGATTAATGAAGCCAATAATTAAGGAGTGATAAAATGCAGGT
  GlnTyrGluAlaPheValIleAsnGluAlaAsnAsn---                 METGlnVal
                                                          Protein III
TTTAGTTTTACCAGAAAATAAGGATATCAATTATATAAAAACGGTCCAAGAAGTAAAACG
 LeuValLeuProGluAsnLysAspIleAsnTyrIleLysThrValGlnGluValLysArg
ATTTTTTGCGGATTTTGAGCGGTTTCGGATGATTACGGGGTTATCAAAAAAGCCACATTT
  PhePheAlaAspPheGluArgPheArgMETIleThrGlyLeuSerLysLysProHisLeu
ACTTAGAAATGGTTTTCTGGAAGAGCCGCAGTTTGAGCCGGTAGCATTTTCTGCTAGACA
  LeuArgAsnGlyPheLeuGluGluProGlnPheGluProValAlaPheSerAlaArgHis
TAATAAAGAAGTCATTTTGGAAGCGCGATGGTTGGTAGAGAAATATACTGAAATGTTGAA
  AsnLysGluValIleLeuGluAlaArgTrpLeuValGluLysTyrThrGluMETLeuAsn
TCAGATGGATGATTTATATCGAACTATTTTGATGGAATGTTACGTGGAACGAAAACAAGA
  GlnMETAspAspLeuTyrArgThrIleLeuMETGluCysTyrValGluArgLysGlnAsp
TGTGGCGGTAATGATGGATTTACCGTATGAAATTGCCCAGTTTAAACGGATAAAAAAACG
  ValAlaValMETMETAspLeuProTyrGluIleAlaGlnPheLysArgIleLysLysArg
GGCAGTGCTAGAACTTGCAACGCTAATGGGGATTTTAGTAAGGAAATGATGATACTTTCG
  AlaValLeuGluLeuAlaThrLeuMETGlyIleLeuValArgLys---
TGATATTTTGAAACATCATTTTCCTATTAATATAGAAGTAAGCTAATTGTCCAGTAAGCG
GATGACAATAAAAGCTGCATCAGAATGAAGGTGCACCGATTTTCTGATAATACATGATGT
TTTACAAGGAATTTGTTTTTATGATTGGATTTAAATCCGTTGAGATAAACAAATATTCTA
TTTTGGAAAGTAAAGTTCGGAGGAATAAATTATTAAATGTGGTCTTGACCGAACTTTGCT
TTCTGTTTTAAAGGAGTGAACGTTTGGTGAAGAGTTTGAGCTTCATGAGAGTTTTGGAAG
                                                  ValLysSerLeuSerPheMETArgValLeuGluAla
                                                                  Protein I
CAGTGAGAACAATGCTCCAGGAAAAAGGCGGACTAGATATTTCTATTGTAATGCGTGACC
   ValArgThrMETLeuGlnGluLysGlyGlyLeuAspIleSerIleValMETArgAspGl
AAGTGGAAATGCCTACAACGATGATCGAGATGATTGATCAAGAGGAAGAAGAAAGCCAAA
  ValGluMETProThrThrMETIleGluMETIleAspGlnGluGluGluGluSerGlnThr
CTGCCTGGAAAGAAAAATACCGTTTTGCAATCCATCATTATACAAATGAAACGGACTTAG
  AlaTrpLysGluLysTyrArgPheAlaIleHisHisTyrThrAsnGluThrAspLeuAla
CGGGAGTCGAAAAGATAGATACGCTTATCCAAACAGGATTCACTTTGCCTGAAGGATACA
  GlyValGluLysIleAspThrLeuIleGlnThrGlyPheThrLeuProGluGlyTyrLys
AATTAATCGCTGTTCGACATTACGGAAAACAAAATTTAGTCAAAGAAAATACGTTAATTC
   LeuIleAlaValArgHisTyrGlyLysGlnAsnLeuValLysGluAsnThrLeuIleHis
ACGCAAAAACCAGTTTTGAAGTAAGTATTTGTCGTGAATTAAAAGTAAAAATTTAGGGGG
  AlaLysThrSerPheGluValSerIleCysArgGluLeuLysValLysIle---
AAATATTAATGGCATTTGAAGAGAATTTATATTGTGATTATACACCGGGAGCTGCTAAAG
          METAlaPheGluGluAsnLeuTyrCysAspTyrThrProGlyAlaAlaLysAla
                Protein II
CGGTCGCGGGGAAAGATGTAATTTTAGCAGTTTTTAACGCAGCGGGGGACAAACTATTAG
  ValAlaGlyLysAspValIleLeuAlaValPheAsnAlaAlaGlyAspLysLeuLeuAla
CGGTTGCGGGCCAACAAGGTCTAACTGTAAACCGTTCTAAAGATAGCATTGAAATTACAT
  ValAlaGlyGlnGlnGlyLeuThrValAsnArgSerLysAspSerIleGluIleThrSer
CTAAAGATACAGTTGGCGGATGGAAATCCAAAATTGGCGGTATGAAAGAATGGTCAATTG
  LysAspThrValGlyGlyTrpLysSerLysIleGlyGlyMETLysGluTrpSerIleGlu
AAAATGACGGATTATATGTCGCTGATGCAGAGTCTCACAAAGAATTGGCGAAATATTTCG
  AsnAspGlyLeuTyrValAlaAspAlaGluSerHisLysGluLeuAlaLysTyrPheGlu
AAAGTGATAGCCCGGTTTGTGTGAAAATCATTAATCAAGCATCTAAAAAAGGTCTTTTCG
  SerAspSerProValCysValLysIleIleAsnGlnAlaSerLysLysGlyLeuPheGly
GTGGTTTGGCAATTGTAGCTGACTATAGTTTTGAAGCACCTTTTGACGAAGCGATGACTT
  GlyLeuAlaIleValAlaAspTyrSerPheGluAlaProPheAspGluAlaMETThrTyr
ACTCTGTAAAACTAGACGGAATGGGCGCGCTTGTTGATTTAACGATTACTGAGGGCGGCG
  SerValLysLeuAspGlyMETGlyAlaLeuValAspLeuThrIleThrGluGlyGlyAsp
ACCAAATGCCCGGCGAAACACCTGTAGCACCAGCAGAATAAAATAGAAAGCCACTGAAAT
  GlnMETProGlyGluThrProValAlaProAlaGlu---
```

AAGTGGCTTTCCCTTAGGAGGAAAATAAATGTTTGAAGTGAATGATACAACTTATATTTT
METPheGluValAsnAspThrThrTyrIleLeu
ACGATTTAATAAACAAAAAGTTAAAACGGTGGAATTAACATCAGGGATTAGTTTAGTTGC
ArgPheAsnLysGlnLysValLysThrValGluLeuThrSerGlyIleSerLeuValAla
AGCTTTGACTGCGAATAAAGGGATTTTGAG
AlaLeuThrAlaAsnLysGlyIleLeu

14. A protein consisting of the amino acid of one of protein I, protein II, and protein III as set forth in sequence:

CTTTTCATTTAGATAAAACAAAAGAAGAAATTGGCGCTTTACCTGCTTCGGCGATTGAAT
PheHisLeuAspLysThrLysGluGluIleGlyAlaLeuProAlaSerAlaIleGluCys
GTCAGTATGAGGCTTTTGTGATTAATGAAGCCAATAATTAAGGAGTGATAAAATGCAGGT
GlnTyrGluAlaPheValIleAsnGluAlaAsnAsn---
METGlnVal
Protein III
TTTAGTTTTACCAGAAAATAAGGATATCAATTATATAAAAACGGTCCAAGAAGTAAAACG
LeuValLeuProGluAsnLysAspIleAsnTyrIleLysThrValGlnGluValLysArg
ATTTTTTGCGGATTTTGAGCGGTTTCGGATGATTACGGGGTTATCAAAAAAGCCACATTT
PhePheAlaAspPheGluArgPheArgMETIleThrGlyLeuSerLysLysProHisLeu
ACTTAGAAATGGTTTTCTGGAAGAGCCGCAGTTTGAGCCGGTAGCATTTTCTGCTAGACA
LeuArgAsnGlyPheLeuGluGluProGlnPheGluProValAlaPheSerAlaArgHis
TAATAAAGAAGTCATTTTGGAAGCGCGATGGTTGGTAGAGAAATATACTGAAATGTTGAA
AsnLysGluValIleLeuGluAlaArgTrpLeuValGluLysTyrThrGluMETLeuAsn
TCAGATGGATGATTTATATCGAACTATTTTGATGGAATGTTACGTGGAACGAAAACAAGA
GlnMETAspAspLeuTyrArgThrIleLeuMETGluCysTyrValGluArgLysGlnAsp
TGTGGCGGTAATGATGGATTTACCGTATGAAATTGCCCAGTTTAAACGGATAAAAAAACG
ValAlaValMETMETAspLeuProTyrGluIleAlaGlnPheLysArgIleLysLysArg
GGCAGTGCTAGAACTTGCAACGCTAATGGGGATTTTAGTAAGGAAATGATGATACTTTCG
AlaValLeuGluLeuAlaThrLeuMETGlyIleLeuValArgLys---
TGATATTTTGAAACATCATTTTCCTATTAATATAGAAGTAAGCTAATTGTCCAGTAAGCG
GATGACAATAAAAGCTGCATCAGAATGAAGGTGCACCGATTTTCTGATAATACATGATGT
TTTACAAGGAATTTGTTTTTATGATTGGATTTAAATCCGTTGAGATAAACAAATATTCTA
TTTTGGAAAGTAAAGTTCGGAGGAATAAATTATTAAATGTGGTCTTGACCGAACTTTGCT
TTCTGTTTTAAAGGAGTGAACGTTTGGTGAAGAGTTTGAGCTTCATGAGAGTTTTGGAAG
ValLysSerLeuSerPheMETArgValLeuGluAla
Protein I
CAGTGAGAACAATGCTCCAGGAAAAAGGCGGACTAGATATTTCTATTGTAATGCGTGACC
ValArgThrMETLeuGlnGluLysGlyGlyLeuAspIleSerIleValMETArgAspGl
AAGTGGAAATGCCTACAACGATGATCGAGATGATTGATCAAGAGGAAGAAGAAAGCCAAA
ValGluMETProThrThrMETIleGluMETIleAspGlnGluGluGluGluSerGlnThr
CTGCCTGGAAAGAAAAATACCGTTTTGCAATCCATCATTATACAAATGAAACGGACTTAG
AlaTrpLysGluLysTyrArgPheAlaIleHisHisTyrThrAsnGluThrAspLeuAla
CGGGAGTCGAAAAGATAGATACGCTTATCCAAACAGGATTCACTTTGCCTGAAGGATACA
GlyValGluLysIleAspThrLeuIleGlnThrGlyPheThrLeuProGluGlyTyrLys
AATTAATCGCTGTTCGACATTACGGAAAACAAAATTTAGTCAAAGAAAATACGTTAATTC
LeuIleAlaValArgHisTyrGlyLysGlnAsnLeuValLysGluAsnThrLeuIleHis
ACGCAAAAACCAGTTTTGAAGTAAGTATTTGTCGTGAATTAAAAGTAAAAATTTAGGGGG
AlaLysThrSerPheGluValSerIleCysArgGluLeuLysValLysIle---
AAATATTAATGGCATTTGAAGAGAATTTATATTGTGATTATACACCGGGAGCTGCTAAAG
METAlaPheGluGluAsnLeuTyrCysAspTyrThrProGlyAlaAlaLysAla
Protein II
CGGTCGCGGGGAAAGATGTAATTTTAGCAGTTTTTAACGCAGCGGGGGACAAACTATTAG
ValAlaGlyLysAspValIleLeuAlaValPheAsnAlaAlaGlyAspLysLeuLeuAla
CGGTTGCGGGCCAACAAGGTCTAACTGTAAACCGTTCTAAAGATAGCATTGAAATTACAT
ValAlaGlyGlnGlnGlyLeuThrValAsnArgSerLysAspSerIleGluIleThrSer
CTAAAGATACAGTTGGCGGATGGAAATCCAAAATTGGCGGTATGAAAGAATGGTCAATTG
LysAspThrValGlyGlyTrpLysSerLysIleGlyGlyMETLysGluTrpSerIleGlu
AAAATGACGGATTATATGTCGCTGATGCAGAGTCTCACAAAGAATTGGCGAAATATTTCG
AsnAspGlyLeuTyrValAlaAspAlaGluSerHisLysGluLeuAlaLysTyrPheGlu

AAAGTGATAGCCCGGTTTGTGTGAAAATCATTAATCAAGCATCTAAAAAAGGTCTTTTCG
SerAspSerProValCysValLysIleIleAsnGlnAlaSerLysLysGlyLeuPheGly
GTGGTTTGGCAATTGTAGCTGACTATAGTTTTGAAGCACCTTTTGACGAAGCGATGACTT
GlyLeuAlaIleValAlaAspTyrSerPheGluAlaProPheAspGluAlaMETThrTyr
ACTCTGTAAAACTAGACGGAATGGGCGCGCTTGTTGATTTAACGATTACTGAGGCCGGCG
SerValLysLeuAspGlyMETGlyAlaLeuValAspLeuThrIleThrGluGlyGlyAsp
ACCAAATGCCCGGCGAAACACCTGTAGCACCAGCAGAATAAAATAGAAAGCCACTGAAAT
GlnMETProGlyGluThrProValAlaProAlaGlu---
AAGTGGCTTTCCCTTAGGAGGAAAATAAATGTTTGAAGTGAATGATACAACTTATATTTT
METPheGluValAsnAspThrThrTyrIleLeu
ACGATTTAATAAACAAAAAGTTAAAACGGTGGAATTAACATCAGGGATTAGTTTAGTTGC
ArgPheAsnLysGlnLysValLysThrValGluLeuThrSerGlyIleSerLeuValAla
AGCTTTGACTGCGAATAAAGGGATTTTGAG
AlaLeuThrAlaAsnLysGlyIleLeu .

15. A process for producing an antibody against Listeria bacteria comprising:

(i) immunizing an animal with a protein which is at least 80% homologous to the amino acid sequence of one of protein I, protein II, and protein III as set forth in sequence:

CTTTTCATTTAGATAAAACAAAAGAAGAAATTGGCGCTTTACCTGCTTCGGCGATTGAAT
PheHisLeuAspLysThrLysGluGluIleGlyAlaLeuProAlaSerAlaIleGluCys
GTCAGTATGAGGCTTTTGTGATTAATGAAGCCAATAATTAAGGAGTGATAAAATGCAGGT
GlnTyrGluAlaPheValIleAsnGluAlaAsnAsn--- METGlnVal
Protein III
TTTAGTTTTACCAGAAAATAAGGATATCAATTATATAAAAACGGTCCAAGAAGTAAAACG
LeuValLeuProGluAsnLysAspIleAsnTyrIleLysThrValGlnGluValLysArg
ATTTTTTGCGGATTTTGAGCGGTTTCGGATGATTACGGGGTTATCAAAAAAGCCACATTT
PhePheAlaAspPheGluArgPheArgMETIleThrGlyLeuSerLysLysProHisLeu
ACTTAGAAATGGTTTTCTGGAAGAGCCGCAGTTTGAGCCGGTAGCATTTTCTGCTAGACA
LeuArgAsnGlyPheLeuGluGluProGlnPheGluProValAlaPheSerAlaArgHis
TAATAAAGAAGTCATTTTGGAAGCGCGATGGTTGGTAGAGAAATATACTGAAATGTTGAA
AsnLysGluValIleLeuGluAlaArgTrpLeuValGluLysTyrThrGluMETLeuAsn
TCAGATGGATGATTTATATCGAACTATTTTGATGGAATGTTACGTGGAACGAAAACAAGA
GlnMETAspAspLeuTyrArgThrIleLeuMETGluCysTyrValGluArgLysGlnAsp
TGTGGCGGTAATGATGGATTTACCGTATGAAATTGCCCAGTTTAAACGGATAAAAAAACG
ValAlaValMETMETAspLeuProTyrGluIleAlaGlnPheLysArgIleLysLysArg
GGCAGTGCTAGAACTTGCAACGCTAATGGGGATTTTAGTAAGGAAATGATGATACTTTCG
AlaValLeuGluLeuAlaThrLeuMETGlyIleLeuValArgLys---
TGATATTTTGAAACATCATTTTCCTATTAATATAGAAGTAAGCTAATTGTCCAGTAAGCG
GATGACAATAAAAGCTGCATCAGAATGAAGGTGCACCGATTTTCTGATAATACATGATGT
TTTACAAGGAATTTGTTTTTATGATTGGATTTAAATCCGTTGAGATAAACAAATATTCTA
TTTTGGAAAGTAAAGTTCGGAGGAATAAATTATTAAATGTGGTCTTGACCGAACTTTGCT
TTCTGTTTTAAAGGAGTGAACGTTTGGTGAAGAGTTTGAGCTTCATGAGAGTTTTGGAAG
ValLysSerLeuSerPheMETArgValLeuGluAla
Protein I

C

CAGTGAGAACAATGCTCCAGGAAAAAGGCGGACTAGATATTTCTATTGTAATGCGTGACC
ValArgThrMETLeuGlnGluLysGlyGlyLeuAspIleSerIleValMETArgAspGl
AAGTGGAAATGCCTACAACGATGATCGAGATGATTGATCAAGAGGAAGAAGAAAGCCAAA
ValGluMETProThrThrMETIleGluMETIleAspGlnGluGluGluGluSerGlnThr
CTGCCTGGAAAGAAAAATACCGTTTTGCAATCCATCATTATACAAATGAAACGGACTTAG
AlaTrpLysGluLysTyrArgPheAlaIleHisHisTyrThrAsnGluThrAspLeuAla
CGGGAGTCGAAAAGATAGATACGCTTATCCAAACAGGATTCACTTTGCCTGAAGGATACA
GlyValGluLysIleAspThrLeuIleGlnThrGlyPheThrLeuProGluGlyTyrLys
AATTAATCGCTGTTCGACATTACGGAAAACAAAATTTAGTCAAAGAAAATACGTTAATTC
LeuIleAlaValArgHisTyrGlyLysGlnAsnLeuValLysGluAsnThrLeuIleHis
ACGCAAAAACCAGTTTTGAAGTAAGTATTTGTCGTGAATTAAAAGTAAAAATTTAGGGGG
AlaLysThrSerPheGluValSerIleCysArgGluLeuLysValLysIle---
AAATATTAATGGCATTTGAAGAGAATTTATATTGTGATTATACACCGGGAGCTGCTAAAG
METAlaPheGluGluAsnLeuTyrCysAspTyrThrProGlyAlaAlaLysAla
→ Protein II
CGGTCGCGGGGAAAGATGTAATTTTAGCAGTTTTTAACGCAGCGGGGGACAAACTATTAG
ValAlaGlyLysAspValIleLeuAlaValPheAsnAlaAlaGlyAspLysLeuLeuAla
CGGTTGCGGGCCAACAAGGTCTAACTGTAAACCGTTCTAAAGATAGCATTGAAATTACAT
ValAlaGlyGlnGlnGlyLeuThrValAsnArgSerLysAspSerIleGluIleThrSer
CTAAAGATACAGTTGGCGGATGGAAATCCAAAATTGGCGGTATGAAAGAATGGTCAATTG
LysAspThrValGlyGlyTrpLysSerLysIleGlyGlyMETLysGluTrpSerIleGlu
AAAATGACGGATTATATGTCGCTGATGCAGAGTCTCACAAAGAATTGGCGAAATATTTCG
AsnAspGlyLeuTyrValAlaAspAlaGluSerHisLysGluLeuAlaLysTyrPheGlu
AAAGTGATAGCCCCGGTTTGTGTGAAAATCATTAATCAAGCATCTAAAAAAGGTCTTTTCG
SerAspSerProValCysValLysIleIleAsnGlnAlaSerLysLysGlyLeuPheGly
GTGGTTTGGCAATTGTAGCTGACTATAGTTTTGAAGCACCTTTTGACGAAGCGATGACTT
GlyLeuAlaIleValAlaAspTyrSerPheGluAlaProPheAspGluAlaMETThrTyr
ACTCTGTAAAACTAGACGGAATGGGCGCGCTTGTTGATTTAACGATTACTGAGGGCGGCG
SerValLysLeuAspGlyMETGlyAlaLeuValAspLeuThrIleThrGluGlyGlyAsp
ACCAAATGCCCGGCGAAACACCTGTAGCACCAGCAGAATAAAATAGAAAGCCACTGAAAT
GlnMETProGlyGluThrProValAlaProAlaGlu---
AAGTGGCTTTCCCTTAGGAGGAAAATAAATGTTTGAAGTGAATGATACAACTTATATTTT
METPheGluValAsnAspThrThrTyrIleLeu
ACGATTTAATAAACAAAAAGTTAAAACGGTGGAATTAACATCAGGGATTAGTTTAGTTGC
ArgPheAsnLysGlnLysValLysThrValGluLeuThrSerGlyIleSerLeuValAla
AGCTTTGACTGCGAATAAAGGGATTTTGAG
AlaLeuThrAlaAsnLysGlyIleLeu ,

under conditions favoring production of antibodies against said protein, and

(ii) purifying said antibodies from said animal.

16. An antibody against Listeria bacteria produced by the process of claim 15.

17. A method for determining Listeria in a sample comprising contacting said sample with the antibody of claim 16, and determining formation of complexes between said antibody and Listeria

bacteria as an indication of Listeria bacteria in said sample.

18. A method for the determination of Listeria bacteria in a sample, wherein the sample is incubated with a nucleic acid as claimed in claim 6, and the label is determined by a suitable detection system.

19. A nucleic acid molecule of claim 10, which hybridizes with an at least 27 nucleotide base section of sequence:

```
CTTTTCATTTAGATAAAACAAAAGAAGAAATTGGCGCTTTACCTGCTTCGGCGATTGAAT
  PheHisLeuAspLysThrLysGluGluIleGlyAlaLeuProAlaSerAlaIleGluCys
GTCAGTATGAGGCTTTTGTGATTAATGAAGCCAATAATTAAGGAGTGATAAAATGCAGGT
  GlnTyrGluAlaPheValIleAsnGluAlaAsnAsn---          METGlnVal
                                                   Protein III
TTTAGTTTTACCAGAAAATAAGGATATCAATTATATAAAAACGGTCCAAGAAGTAAAACG
  LeuValLeuProGluAsnLysAspIleAsnTyrIleLysThrValGlnGluValLysArg
ATTTTTTGCGGATTTTGAGCGGTTTCGGATGATTACGGGGTTATCAAAAAAGCCACATTT
  PhePheAlaAspPheGluArgPheArgMETIleThrGlyLeuSerLysLysProHisLeu
ACTTAGAAATGGTTTTCTGGAAGAGCCGCAGTTTGAGCCGGTAGCATTTTCTGCTAGACA
  LeuArgAsnGlyPheLeuGluGluProGlnPheGluProValAlaPheSerAlaArgHis
TAATAAAGAAGTCATTTTGGAAGCGCGATGGTTGGTAGAGAAATATACTGAAATGTTGAA
  AsnLysGluValIleLeuGluAlaArgTrpLeuValGluLysTyrThrGluMETLeuAsn
TCAGATGGATGATTTATATCGAACTATTTTGATGGAATGTTACGTGGAACGAAAACAAGA
  GlnMETAspAspLeuTyrArgThrIleLeuMETGluCysTyrValGluArgLysGlnAsp
TGTGGCGGTAATGATGGATTTACCGTATGAAATTGCCCAGTTTAAACGGATAAAAAAACG
  ValAlaValMETMETAspLeuProTyrGluIleAlaGlnPheLysArgIleLysLysArg
GGCAGTGCTAGAACTTGCAACGCTAATGGGGATTTTAGTAAGGAAATGATGATACTTTCG
  AlaValLeuGluLeuAlaThrLeuMETGlyIleLeuValArgLys---
TGATATTTTGAAACATCATTTTCCTATTAATATAGAAGTAAGCTAATTGTCCAGTAAGCG
GATGACAATAAAAGCTGCATCAGAATGAAGGTGCACCGATTTTCTGATAATACATGATGT
TTTACAAGGAATTTGTTTTTATGATTGGATTTAAATCCGTTGAGATAAACAAATATTCTA
TTTTGGAAAGTAAAGTTCGGAGGAATAAATTATTAAATGTGGTCTTGACCGAACTTTGCT
TTCTGTTTTAAAGGAGTGAACGTTTGGTGAAGAGTTTGAGCTTCATGAGAGTTTTGGAAG
                                  ValLysSerLeuSerPheMETArgValLeuGluAla
                                                   Protein I
CAGTGAGAACAATGCTCCAGGAAAAAGGCGGACTAGATATTTCTATTGTAATGCGTGACC
   ValArgThrMETLeuGlnGluLysGlyGlyLeuAspIleSerIleValMETArgAspGl
AAGTGGAAATGCCTACAACGATGATCGAGATGATTGATCAAGAGGAAGAAGAAAGCCAAA
  ValGluMETProThrThrMETIleGluMETIleAspGlnGluGluGluGluSerGlnThr
CTGCCTGGAAAGAAAAATACCGTTTTGCAATCCATCATTATACAAATGAAACGGACTTAG
  AlaTrpLysGluLysTyrArgPheAlaIleHisHisTyrThrAsnGluThrAspLeuAla
CGGGAGTCGAAAAGATAGATACGCTTATCCAAACAGGATTCACTTTGCCTGAAGGATACA
  GlyValGluLysIleAspThrLeuIleGlnThrGlyPheThrLeuProGluGlyTyrLys
AATTAATCGCTGTTCGACATTACGGAAAACAAAATTTAGTCAAAGAAAATACGTTAATTC
  LeuIleAlaValArgHisTyrGlyLysGlnAsnLeuValLysGluAsnThrLeuIleHis
ACGCAAAAACCAGTTTTGAAGTAAGTATTTGTCGTGAATTAAAAGTAAAAATTTAGGGGG
  AlaLysThrSerPheGluValSerIleCysArgGluLeuLysValLysIle---
AAATATTAATGGCATTTGAAGAGAATTTATATTGTGATTATACACCGGGAGCTGCTAAAG
        METAlaPheGluGluAsnLeuTyrCysAspTyrThrProGlyAlaAlaLysAla
        Protein II
```

C

```
CGGTCGCGGGGAAAGATGTAATTTTAGCAGTTTTTAACGCAGCGGGGGACAAACTATTAG
  ValAlaGlyLysAspValIleLeuAlaValPheAsnAlaAlaGlyAspLysLeuLeuAla
CGGTTGCGGGCCAACAAGGTCTAACTGTAAACCGTTCTAAAGATAGCATTGAAATTACAT
  ValAlaGlyGlnGlnGlyLeuThrValAsnArgSerLysAspSerIleGluIleThrSer
CTAAAGATACAGTTGGCGGATGGAAATCCAAAATTGGCGGTATGAAAGAATGGTCAATTG
  LysAspThrValGlyGlyTrpLysSerLysIleGlyGlyMETLysGluTrpSerIleGlu
AAAATGACGGATTATATGTCGCTGATGCAGAGTCTCACAAAGAATTGGCGAAATATTTCG
  AsnAspGlyLeuTyrValAlaAspAlaGluSerHisLysGluLeuAlaLysTyrPheGlu
AAAGTGATAGCCCGGTTTGTGTGAAAATCATTAATCAAGCATCTAAAAAAGGTCTTTTCG
  SerAspSerProValCysValLysIleIleAsnGlnAlaSerLysLysGlyLeuPheGly
GTGGTTTGGCAATTGTAGCTGACTATAGTTTTGAAGCACCTTTTGACGAAGCGATGACTT
  GlyLeuAlaIleValAlaAspTyrSerPheGluAlaProPheAspGluAlaMETThrTyr
ACTCTGTAAAACTAGACGGAATGGGCGCGCTTGTTGATTTAACGATTACTGAGGGCGGCG
  SerValLysLeuAspGlyMETGlyAlaLeuValAspLeuThrIleThrGluGlyGlyAsp
ACCAAATGCCCGGCGAAACACCTGTAGCACCAGCAGAATAAAATAGAAAGCCACTGAAAT
  GlnMETProGlyGluThrProValAlaProAlaGlu---
AAGTGGCTTTCCCTTAGGAGGAAAATAAATGTTTGAAGTGAATGATACAACTTATATTTT
                                  METPheGluValAsnAspThrThrTyrIleLeu
ACGATTTAATAAACAAAAAGTTAAAACGGTGGAATTAACATCAGGGATTAGTTTAGTTGC
  ArgPheAsnLysGlnLysValLysThrValGluLeuThrSerGlyIleSerLeuValAla
AGCTTTGACTGCGAATAAAGGGATTTTGAG
  AlaLeuThrAlaAsnLysGlyIleLeu .
```

20. A nucleic acid molecule useful in identifying pathogenic strains of Listeria which hybridizes to a nucleic acid molecule which is complementary to the 2.5kb KpnI-BamHI fragment of plasmid pLM63 (DSM 5220) having the nucleotide base sequence:

```
CTTTTCATTTAGATAAAACAAAAGAAGAAATTGGCGCTTTACCTGCTTCGGCGATTGAAT
  PheHisLeuAspLysThrLysGluGluIleGlyAlaLeuProAlaSerAlaIleGluCys
GTCAGTATGAGGCTTTTGTGATTAATGAAGCCAATAATTAAGGAGTGATAAAATGCAGGT
  GlnTyrGluAlaPheValIleAsnGluAlaAsnAsn---        |METGlnVal
                                                 └ Protein III
TTTAGTTTTACCAGAAAATAAGGATATCAATTATATAAAAACGGTCCAAGAAGTAAAACG
  LeuValLeuProGluAsnLysAspIleAsnTyrIleLysThrValGlnGluValLysArg
ATTTTTTGCGGATTTTGAGCGGTTTCGGATGATTACGGGGTTATCAAAAAAGCCACATTT
  PhePheAlaAspPheGluArgPheArgMETIleThrGlyLeuSerLysLysProHisLeu
ACTTAGAAATGGTTTTCTGGAAGAGCCGCAGTTTGAGCCGGTAGCATTTTCTGCTAGACA
  LeuArgAsnGlyPheLeuGluGluProGlnPheGluProValAlaPheSerAlaArgHis
TAATAAAGAAGTCATTTTGGAAGCGCGATGGTTGGTAGAGAAATATACTGAAATGTTGAA
  AsnLysGluValIleLeuGluAlaArgTrpLeuValGluLysTyrThrGluMETLeuAsn
TCAGATGGATGATTTATATCGAACTATTTTGATGGAATGTTACGTGGAACGAAAACAAGA
  GlnMETAspAspLeuTyrArgThrIleLeuMETGluCysTyrValGluArgLysGlnAsp
TGTGGCGGTAATGATGGATTTACCGTATGAAATTGCCCAGTTTAAACGGATAAAAAAACG
  ValAlaValMETMETAspLeuProTyrGluIleAlaGlnPheLysArgIleLysLysArg
GGCAGTGCTAGAACTTGCAACGCTAATGGGGATTTTAGTAAGGAAATGATGATACTTTCG
  AlaValLeuGluLeuAlaThrLeuMETGlyIleLeuValArgLys---
TGATATTTTGAAACATCATTTTCCTATTAATATAGAAGTAAGCTAATTGTCCAGTAAGCG
GATGACAATAAAAGCTGCATCAGAATGAAGGTGCACCGATTTTCTGATAATACATGATGT
TTTACAAGGAATTTGTTTTTATGATTGGATTTAAATCCGTTGAGATAAACAAATATTCTA
TTTTGGAAAGTAAAGTTCGGAGGAATAAATTATTAAATGTGGTCTTGACCGAACTTTGCT
```

C

under hybridization conditions of 5 - 6 x SSC and 42 - 60°C and after washing for at least 30 minutes at from 0.1-0.5 x SSC and at a temperature from 65°C-70°C.

21. A nucleic acid molecule of claim 20, which is at least 27 nucleotide bases in length.

22. A nucleic acid molecule of claim 20, further comprising a label.

23. A method for determination of Listeria in a sample comprising incubating said sample with a nucleic acid molecule useful in identifying pathogenic strains of Listeria which hybridize to the 2.5kb KpnI-BamHI fragment from plasmid pLM63 (DSM 5220) under hybridization conditions of 5-6 X SSC and 42-60°C and washing at from 65°C to 75°C to a complementary Listeria nucleic acid molecule and determining said hybridization as a determination of Listeria in said sample.

24. Use of the proteins as claimed in claim 13, for the preparation of antibodies against Listeria bacteria.

25. An antibody obtained by immunization of experimental animals with a protein as claimed in claim 13.

26. Use of antibodies as claimed in claim 25, for the immunological determination of Listeria bacteria.

NUCLEOTIDE SEQUENCE OF PLASMID pPLM63

```
10         20         30         40         50         60
CTTTTCATTTAGATAAAACAAAAGAAGAAATTGGCGCTTTACCTGCTTCGGCGATTGAAT
  PheHisLeuAspLysThrLysGluGluIleGlyAlaLeuProAlaSerAlaIleGluCys

70         80         90         100        110        120
GTCAGTATGAGGCTTTTGTGATTAATGAAGCCAATAATTAAGGAGTGATAAAATGCAGGT
  GlnTyrGluAlaPheValIleAsnGluAlaAsnAsn---                METGlnVal
                                                         ↳ Protein III
130        140        150        160        170        180
TTTAGTTTTACCAGAAAATAAGGATATCAATTATATAAAAACGGTCCAAGAAGTAAAACG
 LeuValLeuProGluAsnLysAspIleAsnTyrIleLysThrValGlnGluValLysArg

190        200        210        220        230        240
ATTTTTTGCGGATTTTGAGCGGTTTCGGATGATTACGGGGTTATCAAAAAAGCCACATTT
 PhePheAlaAspPheGluArgPheArgMETIleThrGlyLeuSerLysLysProHisLeu

250        260        270        280        290        300
ACTTAGAAATGGTTTTCTGGAAGAGCCGCAGTTTGAGCCGGTAGCATTTTCTGCTAGACA
 LeuArgAsnGlyPheLeuGluGluProGlnPheGluProValAlaPheSerAlaArgHis

310        320        330        340        350        360
TAATAAAGAAGTCATTTTGGAAGCGCGATGGTTGGTAGAGAAATATACTGAAATGTTGAA
 AsnLysGluValIleLeuGluAlaArgTrpLeuValGluLysTyrThrGluMETLeuAsn

370        380        390        400        410        420
TCAGATGGATGATTTATATCGAACTATTTTGATGGAATGTTACGTGGAACGAAAACAAGA
 GlnMETAspAspLeuTyrArgThrIleLeuMETGluCysTyrValGluArgLysGlnAsp

430        440        450        460        470        480
TGTGGCGGTAATGATGGATTTACCGTATGAAATTGCCCAGTTTAAACGGATAAAAAAACG
 ValAlaValMETMETAspLeuProTyrGluIleAlaGlnPheLysArgIleLysLysArg

490        500        510        520        530        540
GGCAGTGCTAGAACTTGCAACGCTAATGGGGATTTTAGTAAGGAAATGATGATACTTTCG
 AlaValLeuGluLeuAlaThrLeuMETGlyIleLeuValArgLys---
```

Fig. 1A

→ Protein III

```
          550         560         570         580         590         600
          |           |           |           |           |           |
TGATATTTTGAAACATCATTTTCCTATTAATATAGAAGTAAGCTAATTGTCCAGTAAGCG

          610         620         630         640         650         660
          |           |           |           |           |           |
GATGACAATAAAAGCTGCATCAGAATGAAGGTGCACCGATTTTCTGATAATACATGATGT

          670         680         690         700         710         720
          |           |           |           |           |           |
TTTACAAGGAATTTGTTTTTATGATTGGATTTAAATCCGTTGAGATAAACAAATATTCTA

          730         740         750         760         770         780
          |           |           |           |           |           |
TTTTGGAAAGTAAAGTTCGGAGGAATAAATTATTAAATGTGGTCTTGACCGAACTTTGCT

          790         800         810         820         830         840
          |           |           |           |           |           |
TTCTGTTTTAAAGGAGTGAACGTTTGGTGAAGAGTTTGAGCTTCATGAGAGTTTTGGAAG
                                   ValLysSerLeuSerPheMETArgValLeuGluAla
                                                  |
                                                  ↳ Protein I

          850         860         870         880         890         900
          |           |           |           |           |           |
CAGTGAGAACAATGCTCCAGGAAAAAGGCGGACTAGATATTTCTATTGTAATGCGTGACC
   ValArgThrMETLeuGlnGluLysGlyGlyLeuAspIleSerIleValMETArgAspGl

          910         920         930         940         950         960
          |           |           |           |           |           |
AAGTGGAAATGCCTACAACGATGATCGAGATGATTGATCAAGAGGAAGAAGAAAGCCAAA
  ValGluMETProThrThrMETIleGluMETIleAspGlnGluGluGluGluSerGlnThr

          970         980         990         1000        1010        1020
          |           |           |           |           |           |
CTGCCTGGAAAGAAAAATACCGTTTTGCAATCCATCATTATACAAATGAAACGGACTTAG
  AlaTrpLysGluLysTyrArgPheAlaIleHisHisTyrThrAsnGluThrAspLeuAla

          1030        1040        1050        1060        1070        1080
          |           |           |           |           |           |
CGGGAGTCGAAAAGATAGATACGCTTATCCAAACAGGATTCACTTTGCCTGAAGGATACA
  GlyValGluLysIleAspThrLeuIleGlnThrGlyPheThrLeuProGluGlyTyrLys

          1090        1100        1110        1120        1130        1140
          |           |           |           |           |           |
AATTAATCGCTGTTCGACATTACGGAAAACAAAATTTAGTCAAAGAAAATACGTTAATTC
  LeuIleAlaValArgHisTyrGlyLysGlnAsnLeuValLysGluAsnThrLeuIleHis
```

Fig. 1B

```
      1150        1160        1170        1180        1190        1200
       |           |           |           |           |           |
ACGCAAAAACCAGTTTTGAAGTAAGTATTTGTCGTGAATTAAAAGTAAAAATTTAGGGGG
  AlaLysThrSerPheGluValSerIleCysArgGluLeuLysValLysIle---
                                          ——→ Protein I

      1210        1220        1230        1240        1250        1260
       |           |           |           |           |           |
AAATATTAATGGCATTTGAAGAGAATTTATATTGTGATTATACACCGGGAGCTGCTAAAG
  |METAlaPheGluGluAsnLeuTyrCysAspTyrThrProGlyAlaAlaLysAla
  ——→ Protein II

      1270        1280        1290        1300        1310        1320
       |           |           |           |           |           |
CGGTCGCGGGGAAAGATGTAATTTTAGCAGTTTTTAACGCAGCGGGGGACAAACTATTAG
  ValAlaGlyLysAspValIleLeuAlaValPheAsnAlaAlaGlyAspLysLeuLeuAla

      1330        1340        1350        1360        1370        1380
       |           |           |           |           |           |
CGGTTGCGGGCCAACAAGGTCTAACTGTAAACCGTTCTAAAGATAGCATTGAAATTACAT
  ValAlaGlyGlnGlnGlyLeuThrValAsnArgSerLysAspSerIleGluIleThrSer

      1390        1400        1410        1420        1430        1440
       |           |           |           |           |           |
CTAAAGATACAGTTGGCGGATGGAAATCCAAAATTGGCGGTATGAAAGAATGGTCAATTG
  LysAspThrValGlyGlyTrpLysSerLysIleGlyGlyMETLysGluTrpSerIleGlu

      1450        1460        1470        1480        1490        1500
       |           |           |           |           |           |
AAAATGACGGATTATATGTCGCTGATGCAGAGTCTCACAAAGAATTGGCGAAATATTTCG
  AsnAspGlyLeuTyrValAlaAspAlaGluSerHisLysGluLeuAlaLysTyrPheGlu

      1510        1520        1530        1540        1550        1560
       |           |           |           |           |           |
AAAGTGATAGCCCGGTTTGTGTGAAAATCATTAATCAAGCATCTAAAAAAGGTCTTTTCG
  SerAspSerProValCysValLysIleIleAsnGlnAlaSerLysLysGlyLeuPheGly

      1570        1580        1590        1600        1610        1620
       |           |           |           |           |           |
GTGGTTTGGCAATTGTAGCTGACTATAGTTTTGAAGCACCTTTTTGACGAAGCGATGACTT
  GlyLeuAlaIleValAlaAspTyrSerPheGluAlaProPheAspGluAlaMETThrTyr

      1630        1640        1650        1660        1670        1680
       |           |           |           |           |           |
ACTCTGTAAAACTAGACGGAATGGGCGCGCTTGTTGATTTAACGATTACTGAGGGCGGCG
  SerValLysLeuAspGlyMETGlyAlaLeuValAspLeuThrIleThrGluGlyGlyAsp

      1690        1700        1710        1720        1730        1740
       |           |           |           |           |           |
ACCAAATGCCCGGCGAAACACCTGTAGCACCAGCAGAATAAAATAGAAAGCCACTGAAAT
  GlnMETProGlyGluThrProValAlaProAlaGlu---
  ——→ Protein II
```

Fig.1C

```
1750        1760        1770        1780        1790        1800
|           |           |           |           |           |
AAGTGGCTTTCCCTTAGGAGGAAAATAAATGTTTGAAGTGAATGATACAACTTATATTTT
                                   METPheGluValAsnAspThrThrTyrIleLeu

1810        1820        1830        1840        1850        1860
|           |           |           |           |           |
ACGATTTAATAAACAAAAAGTTAAAACGGTGGAATTAACATCAGGGATTAGTTTAGTTGC
 ArgPheAsnLysGlnLysValLysThrValGluLeuThrSerGlyIleSerLeuValAla

1870        1880        1890
|           |           |
AGCTTTGACTGCGAATAAAGGGATTTTGAG
 AlaLeuThrAlaAsnLysGlyIleLeu
```

Fig. 1D