APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1992, p. 2625–2632
0099-2240/92/082625-08$02.00/0

Vol. 58, No. 8

The Homologous and Heterologous Regions within the *iap* Gene Allow Genus- and Species-Specific Identification of *Listeria* spp. by Polymerase Chain Reaction

ANDREAS BUBERT, STEPHAN KÖHLER,† AND WERNER GOEBEL*

Theodor-Boveri-Institut für Biowissenschaften, Lehrstuhl für Mikrobiologie, Universität Würzburg, Würzburg, Germany

Received 26 December 1991/Accepted 5 May 1992

The *iap* gene of *Listeria* species encodes protein p60. The comparison of *iap*-related genes from different *Listeria* species indicated common and variable regions within these genes which appeared to be specific for each *Listeria* species. On the basis of the *iap* gene sequences, pairs of polymerase chain reaction (PCR) primers which allowed the unambiguous identification of all members of the genus *Listeria*, of groups of related *Listeria* species, and of *L. monocytogenes*, exclusively, were selected. The PCR primers specific for *L. monocytogenes* yielded PCR products which represented essentially the repeat region of the *iap* gene. The size of these PCR products allowed an estimate of the number of the TN repeat units within the repeat region of the p60 protein of an *L. monocytogenes* strain. The data indicated that the number of repeat units differed among *L. monocytogenes* isolates.

The genus *Listeria* comprises seven characterized species: *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri*, *L. innocua*, *L. murrayi*, and *L. grayi* (36). Among these *Listeria* species, most of which seem to be widespread in the natural environment, only *L. monocytogenes* is a human pathogen which can cause severe infections, especially in immunocompromised individuals, newborns, and pregnant women (10, 35). Most *L. monocytogenes* infections can be traced back to contaminated food, in particular milk products, where these gram-positive, motile bacteria multiply even at low temperatures (13, 18, 34).

L. monocytogenes belongs to the facultative intracellular bacteria and invades and grows in a variety of mammalian cells, including macrophages, epithelial cells, and fibroblasts (15, 25, 28). The cytolytic toxin listeriolysin has been unequivocally demonstrated to be an essential virulence factor of *L. monocytogenes* which is apparently necessary for the evasion of these parasites from the phagosome into the cytoplasm (2, 16, 20, 39).

Recently, evidence that a metalloprotease and both phosphatidylinositol-specific phospholipase C and another phospholipase C (lecithinase), which are coregulated with listeriolysin, are also virulence factors (8, 17, 26, 29, 30) has been provided.

The transcription of the genes for these proteins is positively regulated together with still other genes by the regulatory protein PrfA (27, 29), which is present in all *L. monocytogenes* strains but absent in the other *Listeria* species (27).

The previously described protein p60 is a major extracellular protein in *L. monocytogenes* which has been suggested to be associated with the invasion of nonprofessional phagocytic cells (24). The gene encoding p60 was therefore designated *iap* (for invasion-associated protein) (23). We have reported recently (22) that p60-related proteins occur in all *Listeria* species. Comparison of the amino acid sequences deduced from the nucleotide sequences of the corresponding *iap* genes demonstrated common and variable regions within the p60 proteins. The variable domains appear to be specific for a given *Listeria* species.

On the basis of these data we have used in this study specific oligonucleotide primers derived from the *iap*-related gene sequences of the different *Listeria* species to carry out polymerase chain reactions (PCR). This procedure allowed the unambiguous identification of all members of the genus *Listeria*, of groups of closely related *Listeria* species, and of *L. monocytogenes* alone.

MATERIALS AND METHODS

Bacterial strains. *L. monocytogenes* Sv1/2a EGD was obtained from S. H. E. Kaufmann, University of Ulm, Ulm, Germany. The rough mutant RIII, derived from a smooth strain of *L. monocytogenes* Sv1/2a, was obtained from J. Potel, Hannover, Germany. All other *Listeria* strains were obtained from the Institute of Medical Microbiology and Hygiene, University of Würzburg, Würzburg, Germany. The *L. monocytogenes* strains belonging to the different serovars were obtained from T. Chakraborty (Würzburg, Germany).

Escherichia coli JM109 and the recombinant plasmid pSK5, which contains the *iap* gene of *L. monocytogenes*, have been described earlier (23). *E. coli* DH5α (23) was used in cloning experiments with the vector pTZ19R.

Media and reagents. *Listeria* strains were cultured in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 37°C. *E. coli* strains were grown in Luria-Bertani broth at 37°C, with ampicillin at a concentration of 50 μg/ml when appropriate.

Restriction enzymes and the random priming labelling kit were purchased from Boehringer Mannheim GmbH, Mannheim, Germany, and used according to the manufacturer's instructions. [α-^{32}P]dATP (3,000 Ci/mmol) was supplied by Amersham International.

DNA isolation. The procedures for isolating *E. coli* plasmid DNA and *E. coli* and *Listeria* chromosomal DNAs were as previously published (23).

Southern blot analysis. Following digestion with *Hin*dIII,

* Corresponding author.
† Present address: INSERM Unité 65, Département "Biologie-Santé," Montpellier, France.

chromosomal and plasmid DNA fragments were separated on a 1% Tris-borate–agarose gel (Tris-borate is 90 mM Tris base, 90 mM H_3BO_3, and 2 mM EDTA [pH 8.0]) and blotted onto a nitrocellulose filter by the method of Southern (38). DNA probes were labelled by the random priming technique (11). Hybridization was carried out in a solution containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's reagent, 0.1% sodium dodecyl sulfate (SDS), 2 mM EDTA (pH 8.0), and 100 μg of denatured salmon sperm DNA per ml in 50% deionized formamide at 40°C for 20 h. The blots were then washed twice in 2× SSC–0.1% SDS at room temperature and then twice (30 min each) in 0.2× SSC–0.1% SDS at 60°C (stringent conditions). Nitrocellulose filters were exposed to X-ray films (Fuji-RXNIF) for 5 to 15 h.

PCR. In order to amplify the entire *iap* gene or portions of it from each *Listeria* species, PCR (32) was performed with various oligonucleotides (see Fig. 3). In general, the reaction volume of 100 μl contained either chromosomal DNA (about 1 μg) or crude bacterial lysate (10^8 bacterial cells heated for 5 min at 110°C), 2.5 U of *Taq* polymerase (Pharmacia) in reaction buffer (10 mM Tris-Cl [pH 8.5], 1.5 mM $MgCl_2$, 50 mM KCl), and 200 μM (each) dGTP, dATP, dTTP, and dCTP. DNA amplification conditions varied in the annealing and elongation step and are therefore explicitly indicated in the figure legends. Each amplification reaction started with an initial denaturation temperature of 94°C for 3 min and was completed with a final elongation step at 72°C for 5 min. For cloning experiments the amplified DNA was purified on a Sepharose CL-6B column (9) and precipitated in NH_4-acetate (2 M final concentration) and isopropanol (50% final concentration).

Cloning and sequencing of PCR products. After purification, the 3′ ends of PCR products were filled in with 1 U of Klenow enzyme in a reaction mixture volume of 20 μl containing 100 μM (each) dGTP, dATP, dTTP, and dCTP, 20 mM Tris-Cl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl, and 1 mM dithiothreitol, for 15 min at 30°C. The reaction was stopped by addition of 1 μl 0.5 M EDTA. The DNA fragments were then phenol treated, precipitated, cleaved with *Sma*I, ligated in the plasmid vector pTZ19R (Pharmacia), and transformed into competent *E. coli* DH5α cells by using standard techniques (33).

DNA sequencing reactions were performed by using the T7 sequencing kit (Pharmacia) with specific primers derived from *iap*-related gene sequences. DNA sequences were analyzed on a VAX computer system by using the Genetics Computer Group sequence analysis software package 6.2 (7).

Nucleotide sequence accession number. The complete sequences of all *iap*-related genes are available under GenBank (Los Alamos, N.Mex.) accession numbers M80349 for the *iap*-related gene of *L. innocua* 6b, M80350 for the *iap*-related gene of *L. ivanovii*, M80352 for that of *L. murrayi*, M80353 for that of *L. seeligeri*, and M80354 for that of *L. welshimeri*.

RESULTS

Identification of species-specific and common regions within the *iap* genes of *Listeria* species by Southern hybridization. Previous studies using the entire *iap* gene as a hybridization probe for genomic DNA from different *Listeria* species indicated the presence of *iap*-related genes in all *Listeria* species (23) except *L. grayi*. Similar results were obtained (Fig. 1A and B) with *iap* gene fragments which were derived from the 5′- and 3′-terminal parts of this gene. In contrast, two fragments from the middle part of the *iap* gene hybridized specifically with genomic DNAs of all *L. monocytogenes* strains tested but not with those of the other *Listeria* species (Fig. 1C and D).

Isolation of *iap*-related genes from the *Listeria* species by PCR, cloning of the PCR products, and determination of their nucleotide sequences. As shown in Fig. 2, the entire *iap*-related genes from all *Listeria* species were amplified by PCR when pairs of oligonucleotide primers derived from the 5′ and 3′ ends of the coding region of the *iap* gene from *L. monocytogenes* (Lis1A and Lis1B [see Fig. 3]) were used. The sizes of the *iap*-related genes thus obtained were estimated by their migration rates in polyacrylamide gels (Fig. 2) and varied from 1.45 kbp for *L. innocua* serotypes 6a and 6b to 1.6 kbp for *L. welshimeri*, *L. seeligeri*, and *L. ivanovii*. The *L. monocytogenes* EGD strain belonging to serotype 1/2a yielded a PCR product of about 1.5 kbp; similar sizes were obtained for the *iap*-related genes of *L. grayi* and *L. murrayi*. The resulting PCR product from each *Listeria* species was cloned in *E. coli* and sequenced. The comparison of all *iap*-related genes showed extended homologies in the 5′- and 3′-terminal parts but highly variable regions in the middle part of these genes (Fig. 3). These sequence data support the hybridization results described above. The amino acid sequences of the corresponding p60-related proteins derived from the *iap*-related genes and the characteristic regions identified in these proteins were recently reported (22).

Selection of PCR oligonucleotide primers for the unambiguous identification of all members of the genus *Listeria*. We tested the two primers Lis1A and Lis1B used in the above-described PCR protocol to determine whether they were suitable for the specific, unambiguous identification of *Listeria* species. For this purpose we carried out PCR with genomic DNAs from a large number of frequently occurring gram-positive and gram-negative bacteria. As shown in Fig. 4, only *Enterococcus faecalis* and *Bacillus cereus* also yielded clear PCR signals with these two primers. The sizes of these latter PCR products were, however, smaller than those obtained with the *Listeria* species. To test whether the gene products encoded by these amplified genes are related to p60 of *L. monocytogenes*, we sequenced 300 nucleotides from the 3′-terminal end of the PCR product of *B. cereus* and derived the amino acid sequence. We did not detect any appreciable homology with the *iap* gene or the p60 protein of *L. monocytogenes* or other *Listeria* species on the nucleotide or on the amino acid sequence level (data not shown).

We next tested another set of primers (UnilisA and Lis1B). UnilisA is derived from the conserved 5′-coding region of the *iap*-related genes. Figure 5 shows that these primers yielded PCR products with all *Listeria* species but not with *E. faecalis* and *B. cereus*. The sizes of the obtained PCR products were as expected and differed only slightly between the group consisting of *L. monocytogenes* and *L. innocua* and the group consisting of *L. ivanovii*, *L. seeligeri*, and *L. welshimeri*, in agreement with the nucleotide sequences of the corresponding genes (Fig. 3). In contrast, the PCR products obtained from *L. murrayi* and *L. grayi* were considerably smaller than expected, probably because of the annealing of the UnilisA primer to the *iap* gene of these two *Listeria* species at an internal sequence other than the anticipated one.

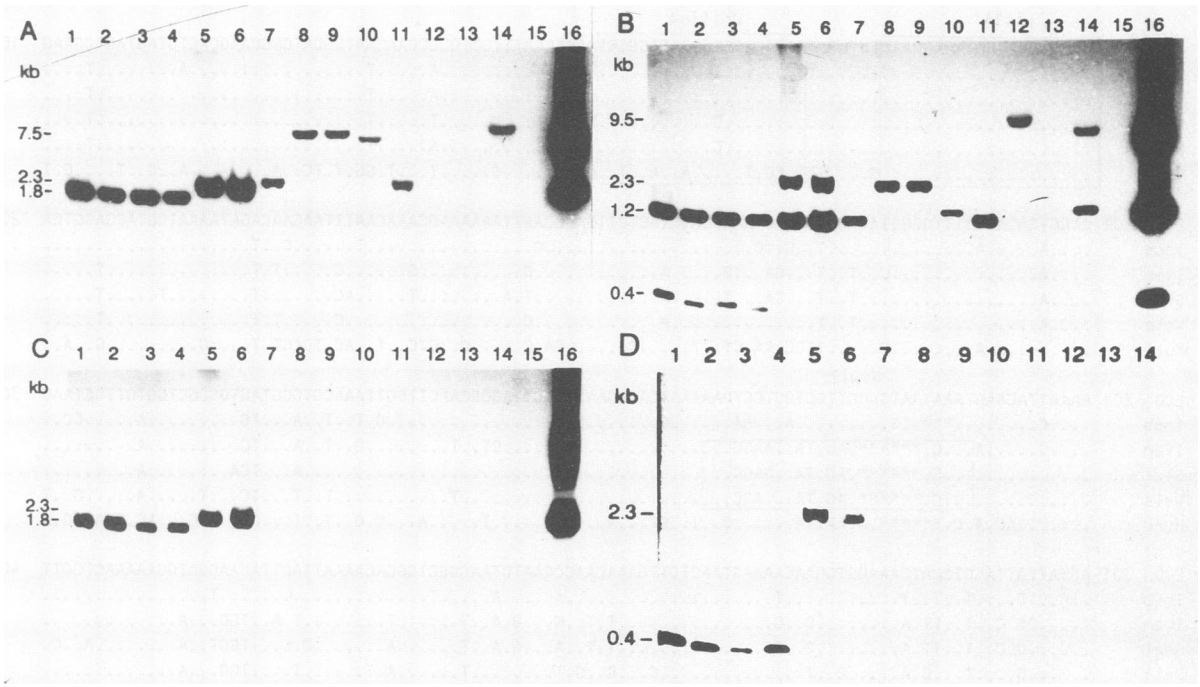


FIG. 1. Detection of hybridizing chromosomal fragments from various *Listeria* strains with four DNA probes derived from the *iap* gene of *L. monocytogenes* EGD. After *Hin*dIII cleavage, DNAs were separated by agarose gel electrophoresis, transferred to a nitrocellulose membrane, and hybridized to the following random priming ^{32}P-labelled *iap* DNA probes: the 580-bp *Dde*I-*Pst*I fragment (5′ end of the gene) (A); the 420-bp *Hin*dIII-*Dde*I fragment (3′ end of the gene) and the 400-bp *Hin*dIII fragment (internal) (B); the 250-bp *Pst*I-*Hin*dIII fragment (internal) (C); and the 400-bp *Hin*dIII fragment (internal) (D). Lanes: 1, *L. monocytogenes* Sv1/2a EGD; 2, *L. monocytogenes* Sv1/2a (SLCC 5764); 3, *L. monocytogenes* RIII (SLCC 5779); 4, *L. monocytogenes* Sv3a (SLCC 5015); 5, *L. monocytogenes* Sv3b (SLCC 5543); 6, *L. monocytogenes* Sv4b (SLCC 4013); 7, *L. welshimeri* A; 8, *L. innocua* Sv6a (NCTC 11288); 9, *L. innocua* Sv6b; 10, *L. ivanovii* (ATCC 19119); 11, *L. welshimeri* B; 12, *L. grayi*; 13, *L. murrayi*; 14, *L. seeligeri*; 15, *E. coli* JM109; 16, *iap*-carrying plasmid pSK5 cut with *Hin*dIII.

PCR primers for the identification of specific groups of *Listeria* species. On the basis of the previously described amino acid sequences of the p60-related proteins obtained from the different *Listeria* species, four groups of *Listeria* species were distinguished (22). The corresponding nucleotide sequences of the *iap*-related genes (Fig. 3) allowed the selection of specific PCR primers for the identification of these four groups. This was performed by changing the 5′-terminal PCR primer (Fig. 3) according to group-specific nucleotide sequences but keeping the downstream PCR primer (Lis1B) constant, as indicated in Fig. 3. This approach is exemplified for *L. innocua* in Fig. 6A and for the *Listeria* group comprising *L. ivanovii*, *L. seeligeri*, and *L. welshimeri* in Fig. 6B.

PCR primers for the specific identification of *L. monocytogenes*. The nucleotide sequences of the *iap* genes and the deduced amino acid sequences of the p60 proteins from two different strains of *L. monocytogenes* both belonging to serotypes 1/2a (strains EGD and Mackaness) were virtually identical in all regions of p60 but differed in the number of the TN repeat units within the repeat region (19 TN repeats in the repeat region of strain EGD and 16 in strain Mackaness). Two PCR primers which were specific for *L. monocytogenes* (Fig. 3) were selected from sequences outside of the repeat region. The expected PCR products should not only be specific for *L. monocytogenes* but should also allow the determination of the number of TN repeat units within the repeat region since these PCR products included the whole repeat region of p60.

Fourteen strains of *L. monocytogenes* belonging to all

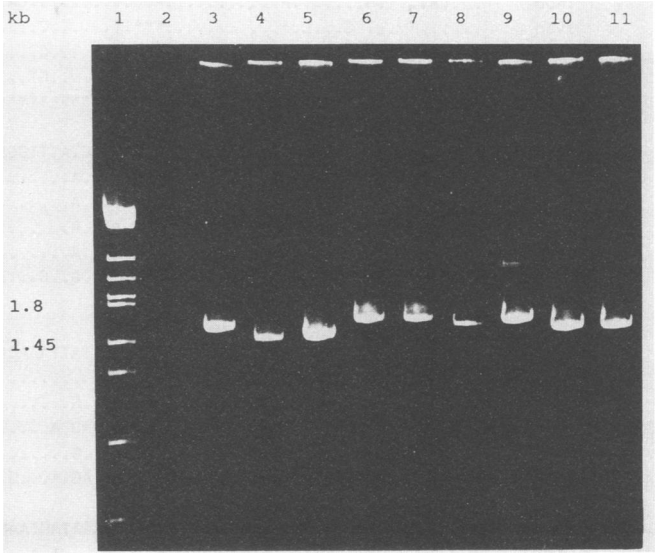
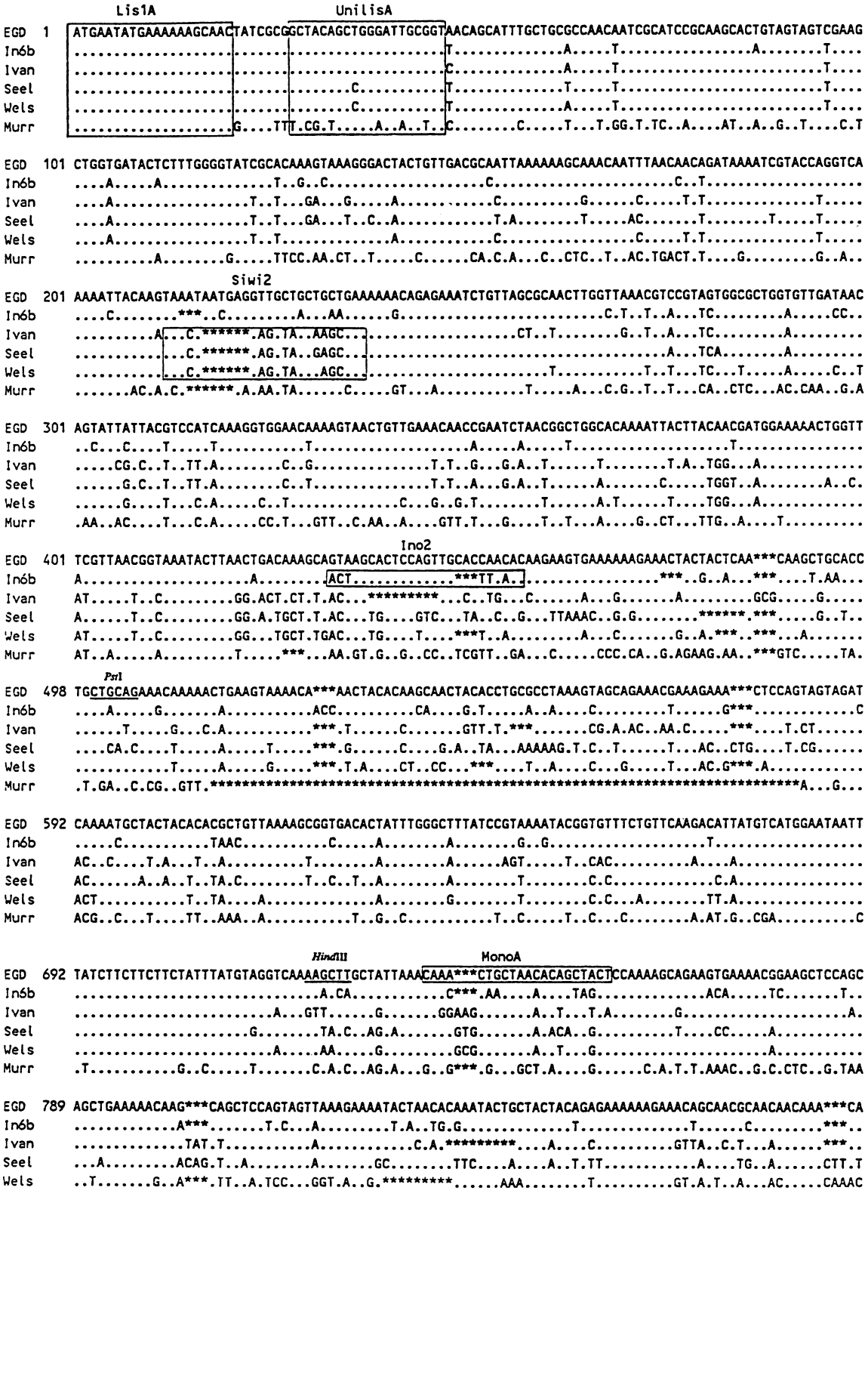


FIG. 2. Genus-specific identification of *Listeria* species by PCR with the oligonucleotide pair Lis1A and Lis1B (see Fig. 3). PCR conditions were as follows: 30 cycles, each at 94°C for 45 s, 50°C for 1 min, and 72°C for 3 min. PCR products were separated on a 4% polyacrylamide gel which was stained with ethidium bromide. Lanes: 1, *Eco*RI-digested *Spp*I DNA molecular mass standard; 2, control reaction (all reaction ingredients except chromosomal DNA); 3, *L. monocytogenes* Sv1/2a EGD; 4, *L. innocua* Sv6a; 5, *L. innocua* Sv6b; 6, *L. welshimeri* A; 7, *L. welshimeri* B; 8, *L. seeligeri*; 9, *L. ivanovii*; 10, *L. grayi*; 11, *L. murrayi*.


Lis1A
UnilisA
Siwi2
Ino2
PstI
HindIII
MonoA
EGD
In6b
Ivan
Seel
Wels
Murr

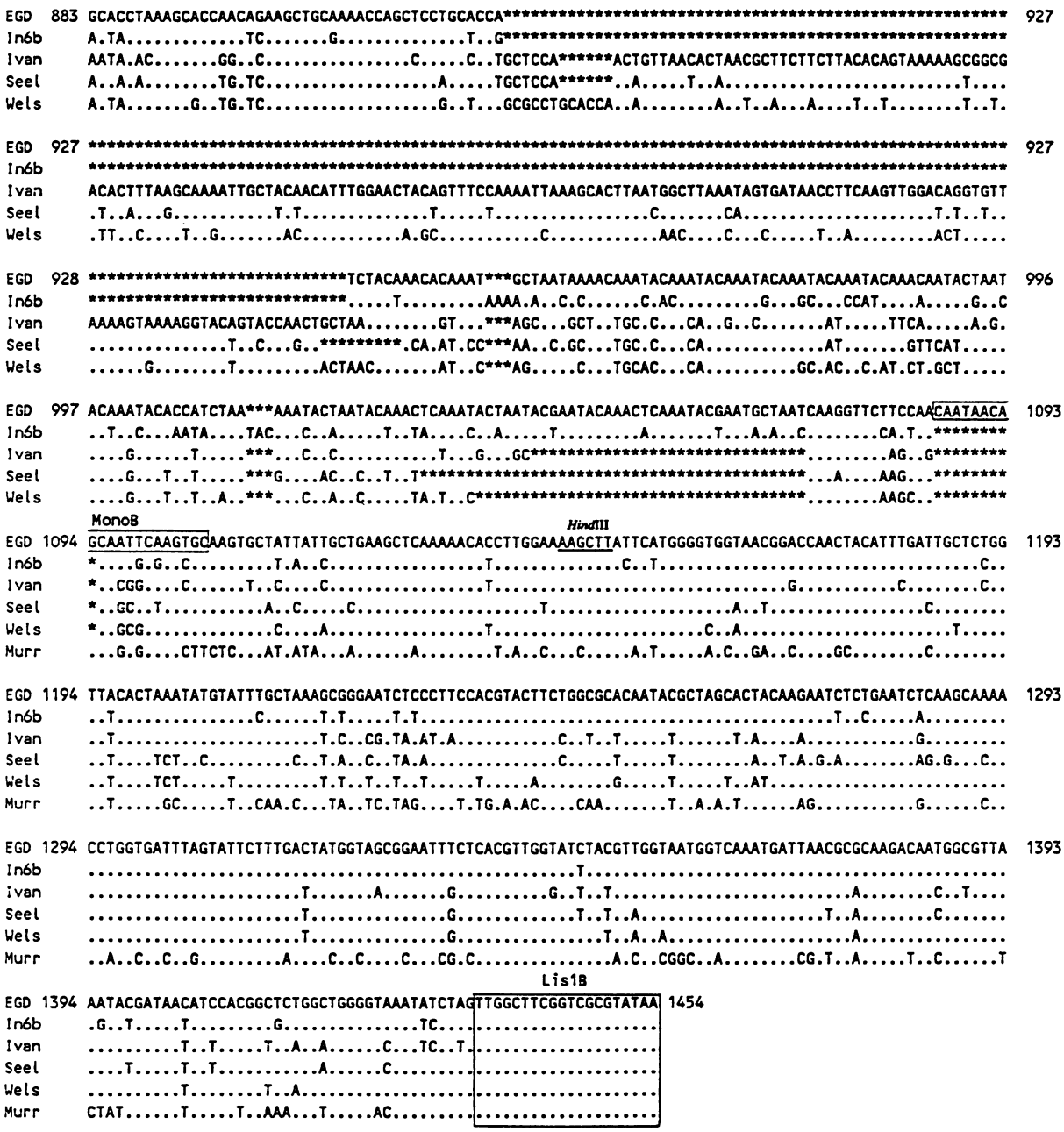


FIG. 3. Comparison of the nucleotide sequences of *iap*-related genes from different *Listeria* species. Each sequence is compared with that of the *iap* gene of *L. monocytogenes* EGD. Dots indicate identical nucleotides; dashes show nucleotide deletions or gaps inserted in order to maintain the highest degree of homology between the compared nucleotide sequences. Oligonucleotide sequences selected for PCR are boxed and titled. The restriction sites for *Pst*I and *Hin*dIII in the *iap* gene are underlined. Abbreviations: EGD, *L. monocytogenes* Sv1/2a EGD; In6b, *L. innocua* Sv6b; Ivan, *L. ivanovii*; Seel, *L. seeligeri*; Wels, *L. welshimeri*; Murr, *L. murrayi* (this sequence shows only the regions with relatively high homology to the other *iap* gene sequences). The complete sequence of the *iap*-related gene of *L. murrayi* is available in GenBank [Los Alamos, N.Mex.]).

known serotypes were tested together with representatives of the other *Listeria* species. As shown in Fig. 7, only the *L. monocytogenes* strains yielded PCR products. The obtained products differed in size by about 0.1 kb. Assuming that the observed size differences exclusively reflected variations in the number of repeat units, the data suggested that, on the basis of the known numbers of repeat units for *L. monocytogenes* EGD (19 units) and Mackaness (16 units), the largest number of repeat units were present in the p60 proteins of the *L. monocytogenes* strains belonging to serotypes 1/2c, 1/2a (EGD), 3c, and 4a/b, and the smallest number were present in the p60 proteins of *L. monocytogenes* strains belonging to serotypes 4a and 4c. The small number of repeat units in p60 of these two serotypes was also confirmed

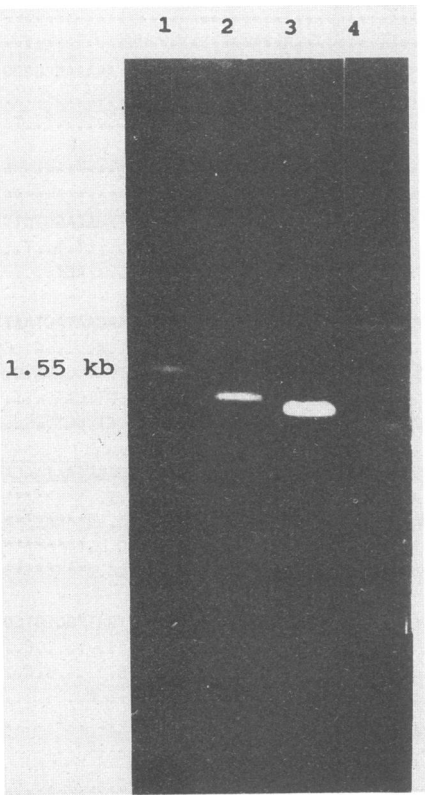


FIG. 4. PCR amplification products obtained by using the oligonucleotide pair Lis1A and Lis1B and lysates from *E. faecalis* (lane 2), *B. cereus* (lane 3), and *Micrococcus flavus* (lane 4). Lane 1, *L. ivanovii*. PCR conditions: 30 cycles, each at 94°C for 45 s and then 55°C for 1 min and 72°C for 3 min.

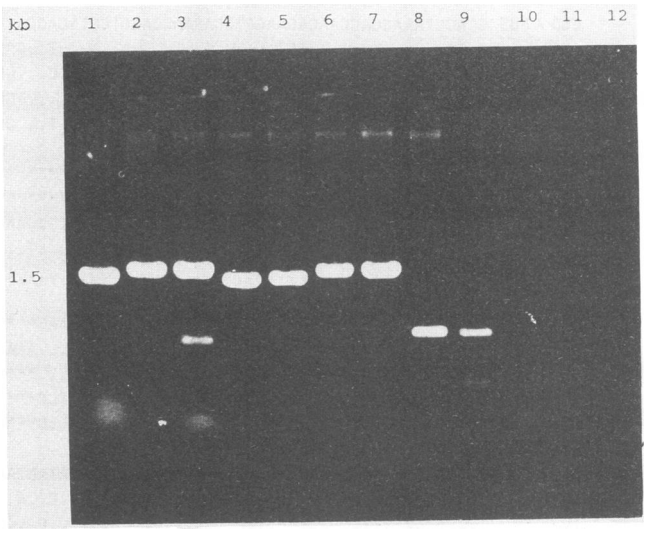


FIG. 5. Genus-specific identification of *Listeria* species by PCR with the oligonucleotide pair UnilisA and Lis1B. PCR conditions were as follows: 30 cycles, each at 94°C for 45 s, 56°C for 30 s, and 72°C for 2 min. PCR products were separated on a 1% agarose gel which was stained with ethidium bromide. Lanes: 1, *L. monocytogenes* Sv1/2a EGD; 2, *L. ivanovii*; 3, *L. seeligeri*; 4, *L. innocua* Sv6a; 5, *L. innocua* Sv6b; 6, *L. welshimeri* A; 7, *L. welshimeri* B; 8, *L. murrayi*; 9, *L. grayi*; 10, *B. cereus*; 11, *M. flavus*; 12, *E. faecalis*.

by direct nucleotide sequence analysis, which yielded 11 repeat units (data not shown). The other *L. monocytogenes* serotypes appeared to contain a number of repeat units similar to that of *L. monocytogenes* 1/2a Mackaness (16 repeat units).

DISCUSSION

The classic microbiological assays, including serotyping and phage typing (10), of *Listeria* species are time-consuming and often not very reliable. More recently, hybridizations with gene probes and synthetic oligonucleotides were employed for the more rapid detection of *Listeria* species. These probes were derived from the genes for listeriolysin O (1, 4, 6, 14), rRNA (1), delayed-type hypersensitivity (DTH) factor (*lmaA*) (31), and p60 protein of *L. monocytogenes* (3, 5, 12, 14, 21, 23).

As shown here, DNA fragments deriving from the 5′- and 3′-terminal regions of the *iap* gene hybridized with all *Listeria* species except *L. grayi* and *L. murrayi*. In contrast, internal *iap* gene fragments hybridized specifically with *L. monocytogenes* only. The hybridization results were in agreement with the nucleotide sequences of the *iap*-related genes from these *Listeria* species which were obtained from all *Listeria* species by PCR with two oligonucleotide primers complementary to the 5′ and 3′ termini of the *iap* gene of *L. monocytogenes*. These *iap*-related genes showed high sequence homology in the 5′ and 3′ regions but considerable variations in the middle parts. The sequence data also explained the specific hybridization of DNA from *L. monocytogenes* isolates with a 500-bp gene fragment (12) and several synthetic oligonucleotides, all of which derive from the middle portion of the *iap* gene.

The comparison of the sequences of the *iap*-related genes allowed the rational design of oligonucleotides for a versatile identification protocol of *Listeria* species by using PCR. Application of two primers from the 5′- and 3′-terminal regions of the *iap* genes yielded PCR products for all *Listeria* species but not for any other bacterial species that were tested. The middle part of the *iap* gene, although highly variable between *L. monocytogenes* and other *Listeria* species, appeared to be constant for a given *Listeria* species or for a group of related *Listeria* species. By fixing the 3′ PCR primer which derived from the common 3′-terminal part of the *iap* genes and varying the 5′ PCR primer according to species-specific sequences from the middle part of the corresponding *iap* gene, we were able to identify *L. monocytogenes* and *L. innocua* separately. *L. seeligeri*, *L. ivanovii*, and *L. welshimeri* showed high homology within the middle part of their *iap* genes, and a PCR primer deriving from this region yielded a PCR product with only this group of *Listeria* species. As our unpublished data showed, the *iap*-related genes from *L. murrayi* and *L. grayi* were highly homologous in this region, which differed from the corresponding regions of the *iap*-related genes of the other *Listeria* species. This allowed again the selection of a 5′ PCR primer which identified these two *Listeria* species only.

As shown previously (23), part of the variable middle section of the *iap* gene of *L. monocytogenes* determines a repeat region which consists of two TN_X repeat domains separated by a PSK motif. This symmetrically arranged repeat domain is virtually absent in all other *Listeria* species. The PCR product generated by the *L. monocytogenes*-specific PCR primers included this entire repeat region. The nucleotide sequences of the *iap* genes from *L. monocyto-*

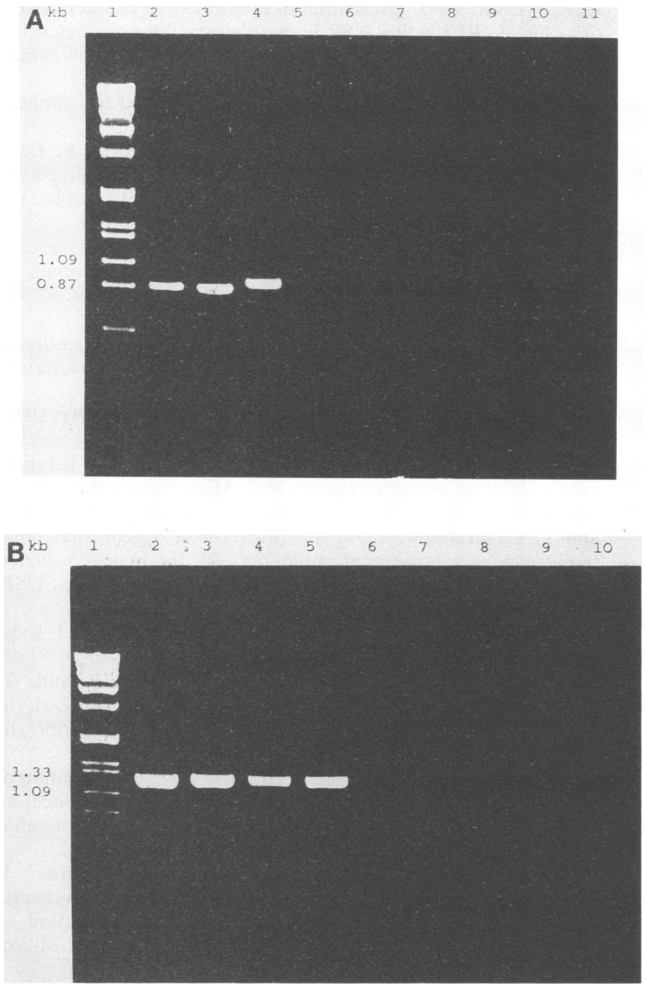


FIG. 6. (A) *L. innocua*-specific PCR products with the oligonucleotide pair Ino2 and Lis1B. PCR conditions were as follows: 30 cycles, each at 94°C for 45 s, 62°C for 60 s, and 72°C for 45 s. Lanes: 1, *Spp*I digested with *Eco*RI; 2, *L. innocua* Sv4ab; 3, *L. innocua* Sv6a; 4, *L. innocua* Sv6b; 5, *L. welshimeri* A; 6, *L. welshimeri* B; 7, *L. ivanovii*; 8, *L. seeligeri*; 9, *L. monocytogenes* Sv1/2a EGD; 10, *L. grayi*; 11, *L. murrayi*. (B) *L. ivanovii*, *L. seeligeri*, and *L. welshimeri* group-specific PCR products with the oligonucleotide pair Siwi2 and Lis1B. PCR conditions were 30 cycles, each at 94°C for 45 s, 58°C for 45 s, and 72°C for 1 min. Lanes: 1, *Spp*I digested with *Eco*RI; 2, *L. ivanovii*; 3, *L. welshimeri* A; 4, *L. welshimeri* B; 5, *L. seeligeri*; 6, *L. innocua* Sv6a; 7, *L. innocua* Sv6b; 8, *L. monocytogenes* Sv1/2a EGD; 9, *L. grayi*; 10, *L. murrayi*.

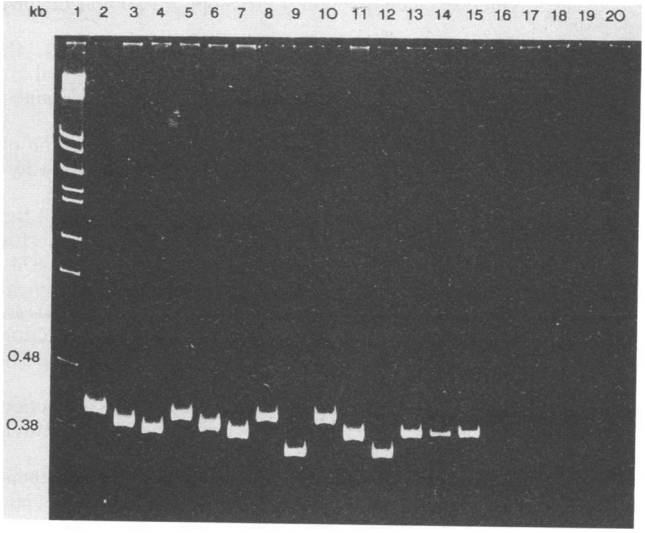


FIG. 7. *L. monocytogenes*-specific PCR products with the oligonucleotide pair MonoA and MonoB. PCR conditions were 30 cycles, each at 94°C for 45 s, 55°C for 30 s, and 72°C for 1 min. PCR products were separated on a 6% polyacrylamide gel which was stained with ethidium bromide. Lanes: 1, molecular size standards; 2, *L. monocytogenes* Sv1/2a EGD; 3, *L. monocytogenes* Sv1/2a Mackaness; 4, *L. monocytogenes* Sv1/2b; 5, *L. monocytogenes* Sv1/2c; 6, *L. monocytogenes* Sv3a; 7, *L. monocytogenes* Sv3b; 8, *L. monocytogenes* Sv3c; 9, *L. monocytogenes* Sv4a; 10, *L. monocytogenes* Sv4ab; 11, *L. monocytogenes* Sv4b; 12, *L. monocytogenes* Sv4c; 13, *L. monocytogenes* Sv4d; 14, *L. monocytogenes* Sv4e; 15, *L. monocytogenes* Sv7; 16, *L. innocua* Sv6a; 17, *L. welshimeri*; 18, *L. seeligeri*; 19, *L. ivanovii*; 20, *L. murrayi*.

genes EGD and *L. monocytogenes* Mackaness were highly homologous in all regions but differed in the number of TN repeat units (19 versus 16). This suggests that the size difference observed in these PCR products obtained with *L. monocytogenes* strains of different serotypes may reflect variations in the number of the repeat units. On the basis of this assumption, one can conclude that the analyzed representatives of the different serotypes differ from *L. monocytogenes* 1/2 Mackaness (16 units) by plus or minus 1, 2, or 3 repeat units, with the exception of the representatives of serotypes 4a and 4c, which seem to possess only 2 or 3 TN repeat units. The rare 4a serotype behaved unusually in other respects as well; e.g., the *lmaA* gene probe, which is highly specific for *L. monocytogenes*, cannot recognize *L. monocytogenes* strains belonging to serotype 4a (31). Furthermore, *L. monocytogenes* strains of this serotype exhibit only low virulence in mice (19). The strain-specific number of TN repeats may be also useful as a characteristic marker of *L. monocytogenes* in epidemiological studies.

The presented data show that the *iap*-related genes from *Listeria* species can be used for the development of a more versatile identification procedure for *Listeria* species by PCR than the reported PCR protocols using mainly the listeriolysin gene (1, 6). Because of the common and variable regions within the *iap*-related genes of the different *Listeria* species, a relatively low number of PCR primers may allow the differentiation of *Listeria* species and possibly even a partial serotyping of *L. monocytogenes* isolates. In contrast to the listerolysin gene, the *iap* gene of *L. monocytogenes* (and probably also the *iap*-related genes of the other *Listeria* species) is essential for cell viability (40) and will be therefore always detectable in the genomes of the *Listeria* species.

ACKNOWLEDGMENTS

We thank M. Wuenscher for helpful discussions and for critically reading the manuscript. E. Appel is thanked for editorial help, and T. Chakraborty is thanked for providing several *L. monocytogenes* strains.

This work was supported by a grant (BMFT KI88059) from the Bundesministerium für Forschung und Technologie.

REFERENCES

1. **Border, P. M., J. J. Howard, G. S. Plastow, and K. W. Siggens.** 1990. Detection of *Listeria* species and *Listeria monocytogenes*

using polymerase chain reaction. Lett. Appl. Microbiol. **11**:158–162.
2. **Cossart, P., M. F. Vicente, J. Mengaud, F. Baquero, J. C. Perez-Diaz, and P. Berche.** 1989. Listeriolysin O is essential for virulence of *Listeria monocytogenes*: direct evidence obtained by gene complementation. Infect. Immun. **57**:3629–3636.
3. **Datta, A. R., B. A. Wentz, and W. E. Hill.** 1987. Detection of hemolytic *Listeria monocytogenes* by using DNA colony hybridization. Appl. Environ. Microbiol. **53**:2256–2259.
4. **Datta, A. R., B. A. Wentz, and J. Russell.** 1990. Cloning of the listeriolysin O gene and development of specific gene probes for *Listeria monocytogenes*. Appl. Environ. Microbiol. **56**:3874–3877.
5. **Datta, A. R., B. A. Wentz, D. Shook, and M. W. Trucksess.** 1988. Synthetic oligodeoxyribonucleotide probes for detection of *Listeria monocytogenes*. Appl. Environ. Microbiol. **54**:2933–2937.
6. **Deneer, H. G., and I. Boychuk.** 1991. Species-specific detection of *Listeria monocytogenes* by DNA amplification. Appl. Environ. Microbiol. **57**:606–609.
7. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
8. **Domann, E., M. Leimeister-Wächter, W. Goebel, and T. Chakraborty.** 1991. Molecular cloning, sequencing, and identification of a metalloprotease gene from *Listeria monocytogenes* that is species specific and physically linked to the listeriolysin gene. Infect. Immun. **59**:65–72.
9. **DuBose, R. F., and D. L. Hartl.** 1990. Rapid purification of PCR products for DNA sequencing using Sepharose CL-6B spin columns. BioTechniques **8**:271–273.
10. **Farber, J. M., and P. I. Peterkin.** 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. **55**:476–511.
11. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specificity. Anal. Biochem. **132**:6–13.
12. **Flamm, R. K., D. Hinrichs, and M. F. Thomashow.** 1989. Cloning of a gene encoding a major secreted polypeptide of *Listeria monocytogenes* and its potential use as a species-specific probe. Appl. Environ. Microbiol. **55**:2251–2256.
13. **Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold.** 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. **312**:404–407.
14. **Furrer, B., U. Candrian, C. Hoefelein, and J. Luethy.** 1991. Detection and identification of *Listeria monocytogenes* in cooked sausage products and in milk by *in vitro* amplification of haemolysin gene fragments. J. Appl. Bacteriol. **70**:372–379.
15. **Gaillard, J.-L., P. Berche, J. Mounier, S. Richard, and P. Sansonetti.** 1987. In vitro model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell-line Caco-2. Infect. Immun. **55**:2822–2829.
16. **Gaillard, J. L., P. Berche, and P. Sansonetti.** 1986. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. Infect. Immun. **52**:50–55.
17. **Geoffroy, C., J. Raveneau, J.-L. Beretti, A. Lecroisey, J.-A. Vazquez-Boland, J. E. Alouf, and P. Berche.** 1991. Purification and characterization of an extracellular 29-kilodalton phospholipase C from *Listeria monocytogenes*. Infect. Immun. **59**:2382–2388.
18. **Gray, M. L., and A. H. Killinger.** 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev. **30**:309–382.
19. **Hof, H.** Unpublished data.
20. **Kathariou, S., P. Metz, H. Hof, and W. Goebel.** 1987. Tn*916*-induced mutations in the hemolysin determinant affecting virulence of *Listeria monocytogenes*. J. Bacteriol. **169**:1291–1297.
21. **Kim, C., L. M. Graves, B. Swaminathan, L. M. Mayer, and R. E. Weaver.** 1991. Evaluation of hybridization characteristics of a cloned pRF106 probe for *Listeria monocytogenes* detection and development of a nonisotopic colony hybridization assay. Appl. Environ. Microbiol. **57**:289–294.
22. **Köhler, S., W. Goebel, and A. Bubert.** Submitted for publication.
23. **Köhler, S., M. Leimeister-Wächter, T. Chakraborty, F. Lottspeich, and W. Goebel.** 1990. The gene coding for protein p60 of *Listeria monocytogenes* and its use as a specific probe for *Listeria monocytogenes*. Infect. Immun. **58**:1943–1950.
24. **Kuhn, M., and W. Goebel.** 1989. Identification of an extracellular protein of *Listeria monocytogenes* possibly involved in intracellular uptake by mammalian cells. Infect. Immun. **57**:55–61.
25. **Kuhn, M., S. Kathariou, and W. Goebel.** 1988. Hemolysin supports survival but not entry of the intracellular bacterium *Listeria monocytogenes*. Infect. Immun. **56**:79–82.
26. **Leimeister-Wächter, M., E. Domann, and T. Chakraborty.** 1991. Detection of a gene encoding a phosphatidylinositol-specific phospholipase C that is co-ordinately expressed with listeriolysin in *Listeria monocytogenes*. Mol. Microbiol. **5**:361–366.
27. **Leimeister-Wächter, M., C. Haffner, E. Domann, W. Goebel, and T. Chakraborty.** 1990. Identification of a gene that positively regulates expression of listeriolysin, the major virulence factor of *Listeria monocytogenes*. Proc. Natl. Acad. Sci. USA **87**:8336–8340.
28. **Mackaness, G. B.** 1962. Cellular resistance to infection. J. Exp. Med. **116**:381–406.
29. **Mengaud, J., S. Dramsi, E. Gouin, J. A. Vazquez-Boland, G. Milon, and P. Cossart.** 1991. Pleiotropic control of *Listeria monocytogenes* virulence factors by a gene that is autoregulated. Mol. Microbiol. **5**:2273–2283.
30. **Mengaud, J., C. Geoffroy, and P. Cossart.** 1991. Identification of a new operon involved in *Listeria monocytogenes* virulence: its first gene encodes a protein homologous to bacterial metalloproteases. Infect. Immun. **59**:1043–1049.
31. **Notermans, S., T. Chakraborty, M. Leimeister-Wächter, J. Dufrenne, K. J. Heuvelman, H. Maas, W. Jansen, K. Wernars, and P. Guinee.** 1989. Specific gene probe for detection of biotyped and serotyped *Listeria* strains. Appl. Environ. Microbiol. **55**:902–906.
32. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Ehrlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
33. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
34. **Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nichols, and C. V. Broome.** 1983. Epidemic listeriosis-evidence for transmission by food. N. Engl. J. Med. **308**:203–206.
35. **Seeliger, H. P. R.** 1961. Listeriosis. Hafner Press, New York.
36. **Seeliger, H. P. R., and D. Jones.** 1986. Genus *Listeria*, p. 1235–1245. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
37. **Sokolovic, Z., et al.** Unpublished data.
38. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
39. **Tilney, L. G., and D. A. Portnoy.** 1989. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite *Listeria monocytogenes*. J. Cell Biol. **109**:1597–1608.
40. **Wuenscher, M., S. Köhler, U. Gerike, and W. Goebel.** Unpublished data.