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# Bacteriophage Typing of *Listeria* Species

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**A bacteriophage typing scheme for differentiating *Listeria* isolates from dairy products and various other foodstuffs was developed. Sixteen selected phages isolated from both environmental sources and lysogenic strains were used for typing and, according to their lytic spectra, divided into four groups. Thus far, 41 distinct patterns of lysis were seen when this set was used in typing 57 defined reference strains, representing all five confirmed species and 16 serotypes in addition to 454 *Listeria* isolates of primarily foodborne origin. Overall, typability was 84.5%; i.e., a strain was lysed by at least one phage at 100× routine test dilution. Strains belonging to serovar 3 were mostly resistant to lysis by the phages employed. The results were highly reproducible, as determined in retyping trials several weeks later. Some phages isolated from environmental sources showed a wider lytic spectrum than did those isolated from lysogenic strains. In accordance with this, the phages were found in different clusters within a computer-generated linkage map. Species specificity and serovar specificity of the lytic reaction were not found. None of the phages was able to lyse strains of *Listeria grayi*, *Listeria murrayi* or *Jonesia denitrificans*. This phage typing system may provide important information for a means of recognizing and eliminating sources of contamination by *Listeria* spp. within dairy plant equipment.**

Dairy products as well as a variety of other foods, the processing of which does not include a final listeriocidal heat treatment, are now widely recognized as possible sources of foodborne listeriosis. Recent outbreaks of the disease (12, 15, 24) emphasized the need for preventative measures in the production and preparation of dairy products, as well as for more refined and accurate typing methods to characterize and discriminate the isolated *Listeria* strains.

Since the contamination of dairy products (especially soft cheeses) by *Listeria* spp. is most often caused by faulty or insufficiently sanitized equipment (29), a factory-specific flora should be found. In order to clearly distinguish those persistent contaminations from sporadic, randomly occurring invasions, isolated *Listeria* strains should be typed beyond the species and serovar levels. Serotyping of *Listeria* is difficult and does not provide sufficient information because of the limited number of serovars among most of the isolated strains. This is especially true for *Listeria monocytogenes* (21).

Molecular typing methods, such as isoenzyme analysis, DNA restriction patterns, and ribosomal RNA analysis, seem to be very promising. However, they are not yet suitable for application in routine typing, especially in smaller laboratories.

Bacteriophages specific for the genus *Listeria* were first described some 45 years ago (25). Since then, a number of phage typing systems for *L. monocytogenes* were developed (2, 9, 16, 20, 27, 30). They are based solely upon phages isolated from lysogenic strains. Some successful applications in epidemiological studies dealing with *L. monocytogenes* were also reported (4, 18, 28). Although attempts have been made to determine and standardize methods used for both the typing procedure and the evaluation of results (22), phages and host strains are not yet available.

The relative occurrence of *L. monocytogenes* in dairy products with respect to the other members of the genus ranges from 14 to 74% (5, 8, 10, 11, 13). However, in our opinion, all five species are of equal value in relation to recognizing sources of contamination as well as in establishing links between contaminated food and the processing equipment involved. Therefore, the aims of this study were to develop a phage typing system suitable for typing not only *L. monocytogenes* but all five *Listeria* species and to further optimize the media and methods used to date.

## MATERIALS AND METHODS

**Culture media.** Tryptose broth (TB), tryptose soft agar (0.4% agar), and tryptose agar (TA, 1.2% agar) were used throughout this study (all from E. Merck AG, Darmstadt, Federal Republic of Germany). However, preliminary investigations revealed that those media could be substantially improved by adding of 1.25 mM $CaCl_2$ per liter of medium as well as adjusting the final pH to 7.00 (data not shown). Those modifications resulted in higher plaque counts and an increased number of phage particles when phage stocks were prepared. All media were autoclaved at 121°C for 15 min. The agar was then dispensed into 100-mm petri dishes at 10 ml per plate to yield a clear, thin, and uniform layer. Soft agar was stored in portions of 4 ml in glass test tubes. Prior to inoculation, it was melted by steaming and placed in a 45°C water bath. All temperatures of incubation were 30°C.

**Bacterial strains.** A selected set of 57 reference strains of *Listeria* spp., including the five confirmed species and all serotypes, was used (Table 1), as were 454 strains isolated from cheese, poultry, and meat as well as from sewage, silage, and the environment. These isolates included 211 strains of *L. monocytogenes* (140 of them were serotyped), 228 strains of *Listeria innocua* (39 were serotyped), 12 strains of *Listeria seeligeri* (8 were serotyped), and 3 strains of *Listeria welshimeri* (none were serotyped). Most strains were isolated during 1988 and 1989 in this laboratory. The procedure was based on a two-step enrichment in listeria

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TABLE 1. Serovar and phagovar types of 57 *Listeria* reference strains tested

| Species | Serovar | Phagovar no. (see Table 3) | Code no. | Strain designation |
|---|---|---|---|---|
| *L. monocytogenes* | 1/2a | 6 | 1040 | SLCC 53, ATCC 15313[a] |
| | 1/2a | 6 | 1005 | SLCC 5634 |
| | 1/2a | 6 | 1006[b] | NCTC 7973, ATCC 35152 |
| | 1/2a | 11 | 1015 | SLCC 5635 |
| | 1/2a | 6 | 1028 | SLCC 5758 |
| | 1/2a | 6 | 1029 | SLCC 5782 (R form) |
| | 1/2a | 6 | 1041 | ATCC 19111 |
| | 1/2a | 6 | 1044 | SLCC 5764 |
| | 1/2b | | 1030 | SLCC 2755 |
| | 1/2c[c] | 1 | 1046 | SLCC 1807 |
| | 1/2c | 6 | 1001[b] | SLCC 2372, ATCC 19112 |
| | 3a | 6 | 1002 | SLCC 2373, ATCC 19113 |
| | 3b | | 1031 | SLCC 1694 |
| | 3c | | 1032[b] | SLCC 2479 |
| | 4a | 11 | 1020 | SLCC 2374, ATCC 19114 |
| | 4d[c] | 11 | 1045 | SLCC 1806 |
| | 4d[c] | 11 | 2047 | SLCC 1090 |
| | 4d[c] | 11 | 1048 | SLCC 1652 |
| | 4a[c] | 38 | 1049 | SLCC 2671 |
| | 4b | 6 | 1003[b] | SLCC 2375, ATCC 19115 |
| | 4b | 6 | 1004 | SLCC 5633 |
| | 4b | 11 | 1016 | SLCC 5636 |
| | 4b | 6 | 1017 | SLCC 5778 (R form) |
| | 4b | 11 | 1039 | ATCC 13932 |
| | 4b | 9 | 1042 | ATCC 23074 |
| | 4c | 37 | 1019[b] | SLCC 2376, ATCC 19116 |
| | 4d | 11 | 1033[b] | SLCC 2377, ATCC 19117 |
| | 4e | 9 | 1018[b] | SLCC 2378, ATCC 19118 |
| | "7" | | 1034[b] | SLCC 2482 |
| *L. innocua* | 4ab | 11 | 2025 | SLCC 2745 |
| | 6a | 41 | 2011[b] | SLCC 3379, ATCC 33090[a] |
| | 6a[c] | | 2054 | SLCC 4286 |
| | 6a | | 2057 | SLCC 5375 |
| | 6a | | 2021 | SLCC 5639 |
| | 6a | 10 | 2022 | SLCC 5656 |
| | 6b | 32 | 2012 | SLCC 3423, ATCC 33091 |
| | 6b | 11 | 2023[b] | SLCC 5640 |
| | 6b | 10 | 2024 | SLCC 5642 |
| | 6b | 11 | 2035 | SLCC 5646 |
| | 6b | 11 | 2051 | SLCC 4211 |
| | 6b | 11 | 2052 | SLCC 4276 |
| | 6b | 11 | 2053 | SLCC 4277 |
| | 6b | 11 | 2055 | SLCC 4295 |
| | 6b | 15 | 2056 | SLCC 5337 |
| *L. ivanovii* | 5 | 36 | 3009[b] | SLCC 4769 |
| | 5 | 36 | 3010 | SLCC 2379, ATCC 19119[a] |
| | 5 | 36 | 3026 | SLCC 5378 |
| | 5 | 36 | 3027 | SLCC 4713 |
| | 5 | 36 | 3050 | SLCC 4719 |
| | 5 | 36 | 3058 | SLCC 3584 |
| | 5 | 36 | 3059 | SLCC 3706 |
| | 5 | 11 | 3060 | SLCC 3765 |
| | 5 | 36 | 3061 | SLCC 3772 |
| | 5 | 36 | 3062 | SLCC 3773 |
| *L. seeligeri* | 1/2b | 6 | 4007[b] | SLCC 3954[a] |
| *L. welshimeri* | 6a | 18 | 50146 | SLCC 7622 |
| | 6b | 22 | 5013 | ATCC 35897[a] |

[a] Type strain.
[b] Used as indicator strain.
[c] Serovar determined is different from the initial one.

enrichment broth (Merck) containing trypaflavine (10 μg/liter) and KSCN (37.5 g/liter) as selective agents. After incubation at 30°C for 24 h (step 1) and 48 h (step 2), a loopful was streaked on both McBride and Oxford agars (Oxoid, Ltd., London, United Kingdom). Plates were evaluated after 24 and 48 h of incubation at 30°C. Suspect *Listeria* colonies were picked for biochemical testing and confirmation of species. This included tests for the production of catalase, motility, Gram stain, hemolytic action (by employing a newly developed tube test which yields reliable results within 3 to 5 h; unpublished data), and fermentation of salicin, rhamnose, and xylose.

Furthermore, *Listeria grayi* (ATCC 19120), *Listeria murrayi* (ATCC 25401), and *Jonesia denitrificans* (ATCC 14870) (one strain of each) were included in this study.

All strains were maintained on TA slants at 4°C and transferred bimonthly.

**Bacteriophages.** First of all, a suitable set of bacterial indicator strains for the isolation and propagation of phages had to be selected (Table 1). It was necessary that the strains be neither lysogenic nor bacteriocinogenic (monocinogenic) (7, 14, 19) and that, furthermore, their colonial shapes be typically smooth rather than rough or otherwise atypical.

A total of 27 phages were initially tested. Of these, 21 were isolated from environmental sources, i.e., sewage effluent from the local sewage treatment plant. Samples were centrifuged at 2,500 × *g* for 10 min to remove any large debris. The supernatant was then filtered through a 0.22-μm-pore-size disposable filter (Syrfil MF, Nucleopore Corp., Pleasanton, Calif.) to yield a sterile solution still containing the presumptive phage particles. Each sample was then mixed with an equal volume of sterile TB and divided into 12 batches (10 ml each). To each was added 0.5 ml of a log-phase culture of one of the potential hosts, i.e., the indicator strains. After 5 to 6 h of incubation in a shaking water bath, the solutions were again centrifuged and filtered. When *Listeria* phages were present in the initial samples, the preparations yielded then contained a sufficient number of phages which could be easily detected by producing plaques on their corresponding host strains in the spot on the lawn method.

Five phages were isolated from lysogenic strains. They were induced and released from the cells by the UV-irradiation technique first described by Lwoff et al. (17). Samples (5 ml) of 2- to 3-h broth cultures of the strains to be examined were dispensed into sterile petri dishes and then exposed to UV light (λ = 254 nm) for 60 s at a distance of 20 cm with a Universal UV lamp (no. 29200, Camag, West Berlin, Federal Republic of Germany). The irradiated cultures were subsequently incubated in the dark for another 3 h and then were centrifuged and filtered. Phage activity was tested for by the spot on the lawn method with the selected indicator strains.

One phage (ATCC 23074-B1) was obtained from the American Type Culture Collection, Rockville, Md.

**Purification, propagation, and titration of phages.** All 27 phages were purified by the soft agar layer method of Adams (1). Serial 10-fold dilutions of the phage-containing suspensions were made and added to tubes containing 4 ml of molten soft agar inoculated with 0.25 ml of log-phase broth cultures of the propagating strains. The mixtures were poured onto TA plates and incubated overnight. A single, well-isolated plaque was then picked from each plate with a sterile Pasteur pipette and placed into 5 ml of TB containing 0.1 ml of a log-phase culture of the host strain. Incubation for 4 h in a shaking water bath was followed by centrifugation and filtration. The procedure was repeated twice to ensure purity of the phage preparations.

In order to prepare high-titer phage stocks, the method of Swanstrom and Adams (26) was used. Double-layered plates containing enough phage to produce nearly confluent lysis

TABLE 2. Bacteriophages and corresponding propagating strains employed

| Bacteriophage | | Propagating strain | | | Plaque diam (mm) | RTD (PFU/ml) |
|---|---|---|---|---|---|---|
| Code no. | Source | Species | Code no. | Serovar | | |
| A513 | Sewage | *L. monocytogenes* | 1001 | 1/2c | 1.5 | $6.0 \times 10^5$ |
| A511 | Sewage | *L. monocytogenes* | 1005 | 1/2a | 1.5 | $5.0 \times 10^5$ |
| A507 | Sewage | *L. monocytogenes* | 1006 | 1/2a | 1.5 | $5.0 \times 10^5$ |
| A502 | Sewage | *L. monocytogenes* | 1006 | 1/2a | 1.0 | $1.0 \times 10^6$ |
| A505 | Sewage | *L. monocytogenes* | 1001 | 1/2c | 1.5 | $2.0 \times 10^6$ |
| A519 | Sewage | *L. monocytogenes* | 1006 | 1/2a | 1.0 | $4.0 \times 10^6$ |
| B604 | Sewage | *L. ivanovii* | 3009 | 5 | 1.5 | $5.0 \times 10^6$ |
| C703 | Sewage | *L. ivanovii* | 3026 | 5 | 1.0 | $1.0 \times 10^7$ |
| B025 | 2025 | *L. ivanovii* | 3009 | 5 | 1.5 | $3.0 \times 10^6$ |
| A528 | Sewage | *L. ivanovii* | 3009 | 5 | 1.5 | $2.0 \times 10^6$ |
| A020 | 1020 | *L. ivanovii* | 3009 | 5 | 1.0 | $6.0 \times 10^6$ |
| B024 | 2024 | *L. ivanovii* | 3009 | 5 | 1.0 | $6.0 \times 10^6$ |
| B012 | 2012 | *L. ivanovii* | 3009 | 5 | 0.5 | $2.0 \times 10^7$ |
| B035 | 2035 | *L. ivanovii* | 3009 | 5 | 1.0 | $6.0 \times 10^6$ |
| C707 | Sewage | *L. ivanovii* | 3009 | 5 | 0.5 | $8.0 \times 10^6$ |
| A500 | ATCC[a] | *L. monocytogenes* | 1042 | 4b | 1.0 | $1.0 \times 10^7$ |

[a] ATCC, American Type Culture Collection.

were prepared for each phage-host system. The overlays were covered with 10 ml of TB, allowed to stand for 10 min, and then scraped off the soft agar with a sterile glass spreader. After removal, the mixtures were extracted for another 30 min at 4°C, centrifuged, and filtered. Sterility of the stock suspensions was tested for by streaking the suspensions on TA plates and incubating them for 48 h.

Each preparation was assayed for PFU with soft agar overlays by using 10-fold dilutions. Counts ranged from $6 \times 10^8$ to $1.2 \times 10^{10}$ PFU/ml, depending on the phage assayed. The routine test dilution (RTD) was determined by the following method. TA plates were flooded with 2 ml of a 4-h TB culture of the host strains. After removal of the excess liquid, the plates were placed in an incubator with the lids removed for at least 30 min in order to achieve dry surfaces. Drops of about 10 μl of 10-fold dilutions of the stocks were placed on the lawns. After overnight incubation, the macroplaques were examined. The highest dilution which just failed to give confluent lysis was defined as the RTD. Since the propagating strains selected were not found to produce monocins or other inhibitory substances which might interfere with typing, we decided to adjust the phage preparations to 100× RTD. This increases the number of typable strains (22; E. P. Guillot and C. S. McCleskey, Bacteriol. Proc., p. 139, 1963). For long-term storage, the phage preparations were supplemented with 10% (vol/vol) sterile glycerol, suspended in amounts of 1.5 ml in sterile Cryotubes (Nunc, Roskilde, Denmark), and further stored at −60°C.

**Phage typing.** Phage typing was performed essentially according to previously published techniques (2, 6). Plates of cultures to be typed were prepared as described above. Small drops of the phage suspensions were then applied, eight per plate, with sterile, disposable micropipettes (Brand, Wertheim, Federal Republic of Germany) with the aid of a hand-held ultramicropipettor (Manostat Corp., New York, N.Y.). Following incubation for 24 and 48 h, lytic reactions were read by placing the plates on a specially designed apparatus emitting oblique transilluminant light (LIS-O1; Dinkelberg, Neu-Ulm, Federal Republic of Germany). Reactions were considered to be positive (more than 10 single plaques up to confluent lysis within one macroplaque) or negative (less than 10 plaques).

**Analysis of results.** Results were computer analyzed on a Cyber 180 computer of the Leibniz Data Processing Center at the Bavarian Academy of Science, Munich, Federal Republic of Germany). The CAVE program (G. Ohmayer, Weihenstephan, Federal Republic of Germany) was used to perform a cluster analysis of the different lytic reactions and, with the obtained data, to generate a linkage map to visualize possible relationships among the phages. This was also done for all *Listeria* strains typed.

## RESULTS

**Bacteriophages.** The phages used in typing, their RTDs, and the corresponding propagating strains are depicted in Table 2. Of the 27 phage suspensions prepared, a set of 16 was selected for typing, since 10 of the 21 phages stemming from sewage were found to be identical to others by plaque morphology and their lytic spectra. The plaque shapes of the phages varied to a considerable degree. Some phages (e.g., A513) produced large plaques which were surrounded by one or more zones of secondary lysis, whereas others (e.g., BO12) gave rise to barely visible plaques with sharp edges. A513, A511, and A507 were the only phages to produce clear plaques with no background growth in the double layer.

When phage preparations were assayed for PFU per milliliter and also when the RTDs were determined, we recognized that some phages needed at least 48 h of incubation for production of clearly visible plaques. Others yielded an increase in PFU of up to 50% after prolonged incubation. Therefore, we recommend that plates prepared for both titration and typing should be incubated for 48 h, although preliminary results may be read after overnight incubation.

The glycerol added to phage preparations did not have any appreciable effect on typing as compared with negative controls.

**Phage typing.** The 41 different patterns of lysis found among all *Listeria* strains typed are shown in Table 3. Phagovars found among the 57 typed reference strains are indicated in Table 1. Phages were divided into four groups, according to their host ranges. The phages of group I revealed a very broad lytic spectrum. They lysed almost all strains which were typable. Group II phages, much more restricted in their host range, lysed primarily members of *L.*

TABLE 3. The 41 lytic patterns (phagovars[a]) detected among 511 *Listeria* strains examined

| Phage Group | Phage Design. | Phagovar type (No.) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | A513 | . | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | . | . | . | . | . |
| | A511 | . | . | . | ■ | ■ | ■ | ■ | ■ | ■ | . | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | . | . | . | . | . |
| | A507 | . | . | . | ■ | ■ | ■ | ■ | . | ■ | . | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | . | . | . | . | . |
| II | A502 | ■ | ■ | . | . | ■ | ■ | ■ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| | A505 | ■ | . | ■ | ■ | ■ | ■ | ■ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| | A519 | ■ | . | . | . | . | ■ | ■ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| III | B604 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | ■ | . | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | . | . |
| | C703 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | . | ■ | . | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | . | . |
| | B025 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | . | . | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | . | . | . |
| | A528 | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | . | . | . |
| | A020 | . | . | . | . | . | . | . | . | . | . | . | . | ■ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | . | . | . | ■ | . | . | ■ | ■ | ■ | . | . |
| | B024 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | ■ | . | . | ■ | ■ | ■ | . | ■ | . | ■ | ■ | ■ | . | . | . | ■ | ■ | ■ | ■ | ■ | ■ | ■ | . | . |
| | B012 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | . | . | ■ | ■ | ■ | . | . | . | . | . | ■ | . | . | . | ■ | ■ | ■ | ■ | ■ | ■ | . | ■ | . |
| | B035 | . | . | . | . | . | . | . | . | . | . | . | ■ | . | . | . | ■ | ■ | . | . | ■ | ■ | . | . | ■ | . | ■ | ■ | ■ | . | ■ | . | . | ■ | ■ | . | ■ | ■ | ■ | . | . |
| | C707 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ | . | . | . | . | . | . | . | . | . | ■ | . | ■ | . | . | . | ■ | . | . | ■ | ■ | ■ | ■ | ■ | . | . |
| IV | A500 | . | . | . | . | . | . | ■ | ■ | ■ | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | ■ |

[a] Symbols: ■, lysis; ·, no reaction.

*monocytogenes*. The phages of group III were most useful for discrimination, since their host range was relatively narrow. All temperate phages belonged to this group, whereas groups I and II were composed solely of environmental phages. However, all group III phages were able to lyse members of each *Listeria* species tested, with the exception of *L. seeligeri*, of which none of the 13 strains tested was lysed by phages B012, B035 or C707. Our decision to place phage A500 in its own group (group IV) was based upon its extremely restricted host range. Few strains were attacked, and those which were attacked were either *L. monocytogenes* or *L. innocua*.

With respect to the relative frequency of occurrence, phagovar 11 ranked first (representing 29% of all strains examined), followed by phagovars 6 (9%) and 4 (7%). Most other phagovars were found among 5 to 10 different strains typed. However, some phagovars (e.g., phagovars 1, 5, 37, 38, 39, and 40) could be assigned to a single strain only.

The results of the typing of 511 strains of *Listeria* spp. with respect to species and serovars are presented in Table 4. Strains resistant to the phages employed in this set belonged primarily to serovar 3 of *L. monocytogenes* and *L. seeligeri*, as well as to serovar 6a of *L. innocua*. Strains of serovar 5, i.e., of *L. ivanovii*, were characterized by their sensitivity to

TABLE 4. Typability of 511 *Listeria* strains with respect to species and serovar

| Serovar | No. strains typable/no. tested | | | | |
|---|---|---|---|---|---|
| | *L. monocytogenes* | *L. innocua* | *L. ivanovii* | *L. seeligeri* | *L. welshimeri* |
| 1/2a | 58/61 | | | 1/1 | |
| 1/2b | 27/34 | | | 1/1 | |
| 1/2c | 9/9 | | | | |
| 3a | 1/7 | | | | |
| 3b | 0/9 | | | 0/1 | |
| 3c | 1/4 | | | | |
| 4a | 1/1 | | | | |
| 4ab | 4/4 | 2/2 | | 1/1 | |
| 4b | 33/34 | | | 0/1 | |
| 4c | 2/2 | | | 1/1 | |
| 4d | 1/1 | | | | |
| 4e | 1/1 | | | | |
| 5 | | | 10/10 | | |
| 6a | | 30/39 | | | 1/1 |
| 6b | | 32/34 | | 2/3 | 1/1 |
| "7" | 0/1 | | | | |
| ND[a] | 51/71 | 155/169 | | 3/4 | 3/3 |
| Mean (%)[b] | 189/239 (79%) | 219/244 (90%) | 10/10 (100%) | 9/13 (70%) | 5/5 (100%) |

[a] ND, Not determined.
[b] Total strains typable, 432 of 511 (84.5%).

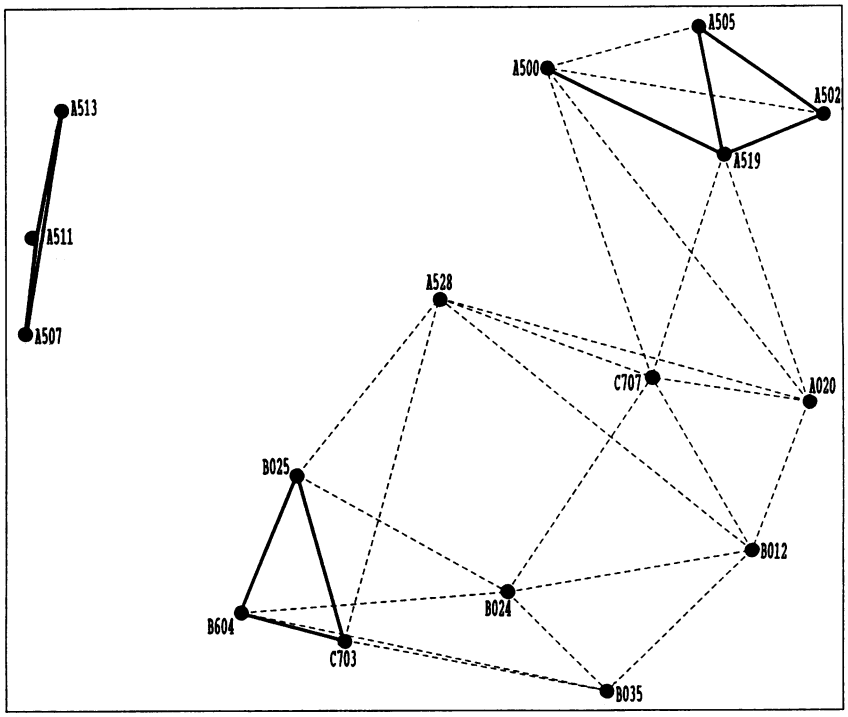


FIG. 1. Linkage map showing the levels of similarity among the phages included in the typing set. Symbols: ——, one or two differences in the lytic pattern; ----, three differences in the lytic pattern.

group III phages. All but one of the tested strains were lysed by all phages of this group. Phage susceptibility was highest (96%) among strains of serovar 4. Overall typability was 84.5%.

*L. grayi*, *L. murrayi*, and *J. denitrificans* (formerly *Listeria denitrificans*) were not lysed by any of the phages.

To assess the reproducibility and reliability of the lytic patterns, retyping trials were carried out several weeks after the initial typing. A set of 60 randomly selected strains was phage typed again by the exact same technique as was used previously. However, typing was performed by another person in order to avoid any bias. The results obtained were identical to those yielded in the initial typing, except for two strains of *L. innocua*, for which formerly positive reactions by phage A020 or B024 could not be confirmed. Those strains were thus considered resistant to these phages.

**Computer analysis of data.** The host ranges found among the phages served as the basis for a computer-generated linkage map (Fig. 1).

When the *Listeria* strains representing all 41 phagovars were analyzed for correlations, no appreciable clusters of species or serotypes could be observed. Nor was a correlation seen between the source of isolation and phagovar. However, isolates from meat and poultry revealed higher degrees of resistance than did strains stemming from cheese and other dairy products.

## DISCUSSION

In this investigation, we attempted to develop a phage typing system for subdividing isolated *Listeria* strains beyond the species and serovar levels. Among the five species included in this study, *L. monocytogenes* and *L. innocua* account for more than 95% of all strains routinely isolated from dairy products, with *L. innocua* being the most prevalent. While this species can be subdivided into three serovars, two of which (6a and 6b) are most often found, we were able to distinguish 23 phagovars among the 244 strains tested. Regarding *L. monocytogenes*, 4 serovars (1/2a, 1/2b, 1/2c, and 4b) are most common among the 13 possible, and 14 phagovars were observed among the 239 strains tested.

Typability was 92% for serovars 1/2 and 4. This compares favorably with the results reported by other workers: depending on the number of strains examined and the phages used for typing, the proportion of phage-typable strains of these serovars ranged from 52 (22) to 78% (2). The most recent data published describes 61% typability found for *L. monocytogenes* serogroup 1/2 and 4 strains within 2,470 strains tested (3). Employing 11 phages isolated from *L. innocua* and *L. ivanovii* lysogenic strains without induction, Rocourt et al. (23) were able to type 92.1% of serovar 5 strains and 61.7% of *L. innocua* strains.

Fundamental differences have been noted with respect to the species and serovar specificity of the lytic action. Audurier and Martin (3), Rocourt et al. (22), and Ortel (20) reported very high specificities of the phages, since no lytic reactions on other species in the genus *Listeria* were observed. Furthermore, the lytic spectrum of each phage was restricted to strains of the parent serovar of *L. monocytogenes*. However, none of our phages was restricted to one species or serovar in its lytic reaction. These contrary results may have been partly due to the different sources of phages, since environmental phages were determined to be far less specific than those isolated from lysogenic strains.

Another factor of error might have been the determination of serovars. When we ordered strains from the Special Listeria Culture Collection, Wuerzburg, Federal Republic of

Germany, which were found to carry prophages (2, 18, 23), they were serotyped again prior to shipment. However, in 6 of 13 strains, a serovar different from the initial one was determined (Table 1). It is apparently not unusual that serotyping of strains which have been stored for a while yields deviating results (A. Schwarzkopf, Wuerzburg, Federal Republic of Germany, personal communication).

With respect to serovar 1/2 strains, however, some specificity was observed, because they were lysed only by phages which were propagated on serovar 1/2 strains. Since those phages could be propagated on a variety of *Listeria* host strains, an intriguing task would be to determine their host ranges after propagation on different hosts.

In an early study in 1963, Guillot and McCleskey (Bacteriol. Proc. 1963) also found little or no correlation between the serovar source of phages and the serovar of susceptible hosts. A total of 40 phages and 123 strains of *Listeria* spp. were involved in their investigation.

Another noteworthy fact is the typability of the two rough strains, which were attacked by group I and group II phages. Those included in group I in particular are by no means related to the others, as visualized in Fig. 1. These findings are supported by studies of the morphologies, as well as the molecular compositions of the phage particles, which are presently being conducted.

It remains somewhat unclear as to why strains belonging to the rarely isolated serovar 3 were primarily resistant to phage-induced lysis. This observation was also reported by all other workers in this area. Our efforts to isolate temperate phages from those strains were also unsuccessful.

More phages have to be isolated in order to further increase the typability and to subdivide the large portion of strains characterized by phagovar 11. We aim to carry out such investigations.

However, our present study indicates that the use of phage typing is a quite promising means of discriminating and differentiating isolated *Listeria* strains. The usefulness of this method with respect to distinguishing persistent contaminations within dairy plants from sporadically occurring impacts of *Listeria* spp. was recently underscored in some cases involving dairies located in West Germany as well as in Austria. The obtained results will be reported separately.

As soon as the reliability of the system is sufficiently confirmed, it will be made available for general use.

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