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Inducing L Forms in *Listeria monocytogenes* Types 1 Through 7

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Several investigators (M. Suchanova and F. Patochka, Czech. Epidemiol. Mikrobiol. Immunol. **6**:133–139, 1957; Zentr. Bakteriol. Parasitenk. Abt. I Orig. **170**:547–564, 1958) have postulated the existence of a filterable or L form of *Listeria monocytogenes*. Their work did not describe the morphology or method of induction of the L form, or the reversion process which can take place. We have developed an easy, reliable method to induce L forms in the seven serotypes of *L. monocytogenes*, and our results both support past implications and provide a tool for further experimentation on various aspects of the organism's intriguing pathogenesis.

The medium (L-medium) utilized in this study to induce and maintain *L. monocytogenes* L forms contained the following ingredients per 100 ml: N I H Thioglycollate Broth (Difco), 4.0 g; sucrose (0.5 M), 17.0 g; Difco Special Agar (Noble), 0.8 g; and penicillin G, 6,000 units. It was prepared by autoclaving the broth and agar combined in 50 ml of distilled water. Sucrose, sterilized at 2 × concentration at 121 C for 10 min, and penicillin (600 units/ml) were added after the medium cooled to bring the total volume to 100 ml.

Organisms utilized as routine inocula were grown 18 to 24 hr at room temperature on Trypticase Soy Agar (BBL) slants, and suspensions were made in 10 ml of PPLO Enrichment Broth (Difco). A 0.1- to 0.2-ml amount of the suspension was inoculated into the L-medium by pour plating or was spotted on the surface.

Formation of L forms was consistent in most old stock and freshly isolated cultures of *L. monocytogenes* at room temperature incubation. The times required for the transformation varied markedly among the serotypes.

L forms were obtained from both surface and pour platings. Granular growth, which developed into typical L colonies for most serotypes, appeared in 18 hr on surface platings which had been covered with a sterile glass microscope slide. Pour platings had typical L forms distributed throughout the medium in 5 to 8 days.

Colonial structure varied slightly among the serotypes; it was basically a dark center with a medium to large periphery, moderately granulated and vacuolated.

Horse serum (1 to 20% concentrations) and a 10% CO_2 atmosphere had no effect on L-form induction or maintenance.

The L forms were successfully subcultured by methods described as standard for L-form work. Agar blocks containing surface L colonies were pushed face down across fresh medium. Large colonies appeared along the line of inoculation and where the block was left on the new medium. Smaller colonies grew away from the line of streaking.

Several agar blocks containing L-form colonies were inoculated into the L-medium prepared as a broth. After incubation, the agar blocks became covered with L-form growth, with subsequent spreading into the medium. Moderate shaking during incubation was helpful in developing L forms in liquid culture rapidly.

With the procedure outlined, types 2, 4, 6, and 7 of *L. monocytogenes* reverted from the L form to the classical bacillary morphology in 18 to 20 hr on penicillin-free thioglycolate medium and on blood agar. In the absence of inducing agents, types 1, 3, and 5 remained in the L form.