Comparison of Temperature and pH Effects on Cells, Walls, and Autolysin of *Listeria monocytogenes*[1] (37488)

ELIZABETH A. TYRRELL, SUSAN M. BRANDE, AND P. C. RAJAM
Department of the Biological Sciences, Smith College, Northampton, Massachusetts 01060

We have shown that isolated cell walls of *Listeria monocytogenes* Strain A4413 are labile (1), thus extending an earlier demonstration of wall lability in two other strains of this organism (2). Lability is not confined to walls, since cells of A4413 and of 18 additional *Listeria* strains autolyze spontaneously in culture (3). Thus an unusual number of lytic strains within one species is available for study of contemporary theories of wall maintenance.

This report demonstrates wall lability in one such strain, and the release of autolysin by these walls. Temperature and pH effects on cellular autolysis, wall dissolution, and autolysin activity are compared.

Materials and Methods. Organism. The derivation of *L. monocytogenes* Strain 5 has been described, as have conditions of growth (3). In brief, organisms were grown in brain–heart infusion broth at 35° on a reciprocating shaker, growth and subsequent lysis being followed by turbidimetry at 655 nm (1, 3). Under these conditions, cultures reached peak density in 9 to 10 hr, when culture pH had stabilized at 6.3.

Cell walls. Strain 5 walls were prepared as previously described (1) by sonication of cells from cultures at peak density. Between 30 and 40 ml of washed walls at optical density (OD_{655}) 0.3 were obtained from 1.5 liters of culture. Dissolution rates of wall suspensions were studied as described below. Inactivated walls (for use as autolysin substrate) were prepared by heating fresh walls in distilled water suspension, at 65 to 68° for 10 min. Inactivated walls were adjusted to OD_{655} 0.6.

Autolysin. This term applies to the mixture of degradative enzymes obtained in solution following complete dissolution of fresh wall suspensions. Dissolution (initial OD_{655} 0.3) was accomplished in Tris–HCl buffer (0.5 *M*, pH 7.5, 35°). Centrifugation (37,000*g*, 10 min, 2°) yielded clear supernatant fluid which was used undiluted as a source of autolysin. Two batches (Lots 1 and 2) differing slightly in activity and kinetics were used in these experiments at equivalent total protein (4) concentration (385 μg/ml). Autolysin was assayed by mixing equal volumes of autolysin and inactivated substrate (final OD_{655} about 0.3) at 0°, followed by incubation at the desired temperature or pH. Kinetics were followed by turbidimetry.

Adjustments in temperature and pH. In general, each of the above systems was studied over a range of pH at a constant 35 ± 0.1°; or over a range of temperature at a constant pH. Cultures at peak density (35°, pH 6.3) were adjusted to the desired pH with 0.1 *N* HCl or NaOH, using acid or base volumes too small to affect optical density. Incubation was continued at 35°. Alternatively, peak density cultures at pH 6.3 were rapidly equilibrated to temperatures above or below 35°. Wall suspensions in appropriate buffers between pH 5.0 and 8.0 were incubated at 35°; or incubated at alternate temperatures in the pH range 7.7 to 7.0. Autolysin solutions were incubated with inactivated wall substrate between pH 5.0 and 9.0 at 35°, or at alternate temperatures at pH 7. Buffers used are described in the appropriate figure legends.

Results. Immediately following peak growth, Strain 5 exhibited an autolytic curve composed of three distinct sequential rates, confirming an earlier finding (3). Adjustment to pH 7 or 8 resulted in progressive

[1] Supported in part by an award from the Lalor Foundation.

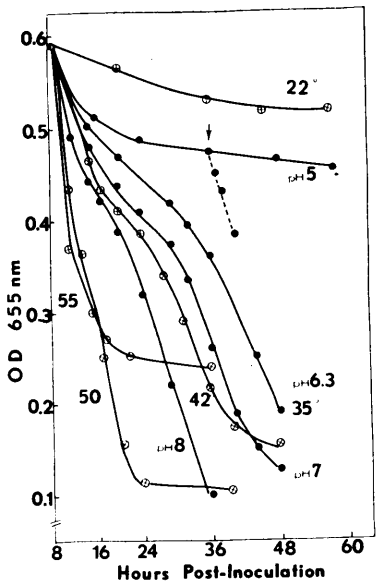

FIG. 1. Temperature and pH effects on cell lysis. Cultures (35°, pH 6.3) were adjusted in pH (●) or held at pH 6.3 at different temperatures (⊗). Arrow, readjustment of pH 5 cultures to pH 7; resulting rate change (- - -). Curve at 35°, pH 6.3 shows the normal kinetics of Strain 5 in culture. Points represent average readings from triplicate cultures. Maximum variation, 0.03 OD units.

increase in overall lytic rate, the major effect being exerted on the first and second rates. Adjustment to pH 5 largely negated the second and third rates, with minimal effect on the initial drop in optical density. Even after 27 hr at pH 5, readjustment to pH 7 reversed stasis.

At 22° (pH 6.3) normal autolytic behavior was largely negated. Above 35° major increases in overall autolytic rates occurred; however, premature cessation of lysis was noted at 55°. These, and the above findings (Fig. 1) suggested an enzymatic base for the autolytic curve.

Wall dissolution rates (Fig. 2) were maximum at pH 7 and minimum at pH 5 (35°), and were favored by Tris as opposed to phosphate buffers. Rates were minimum at 20° and markedly increased up to 50° where dissolution was complete in 30 min. Walls were inactivated in 5 min at 68°.

Autolysin activity was maximum at pH 8 (35°) and minimum at pH 5 (Fig. 3). Activity was minimum at 21°, increasing sharply with temperature. However, increase in activity appeared to be balanced with inactivation as a function of time. Autolysin was inactivated in 5 min at 70° (Fig. 4).

Discussion. Contemporary theories of wall maintenance eventually refer autolysis to the action of degradative enzymes (autolysins) on cell wall substrate. It follows that overall rates of cell lysis, wall dissolution, and free autolysin activity should be similarly affected by temperature and pH. Despite differences in ionic environments and concentrations among our several systems, qualitatively similar effects were obtained, thus confirming the theoretical prediction.

Rate changes during cellular autolysis suggest that at least three events take place. These may involve temporal differences in dissolution of distinct wall areas and/or activity of multiple autolysins (1). The differential effects of temperature and pH on cellular autolytic rates are consistent with

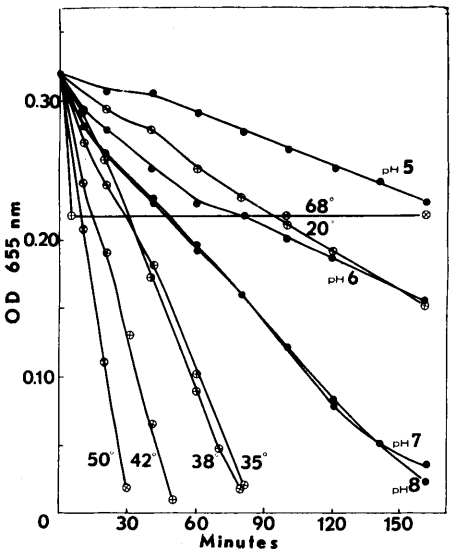

FIG. 2. Temperature and pH effects on wall dissolution. Walls were suspended in buffers (●) at pH 5 (0.05 *M*, succinic acid–NaOH), 6, 7, or 8 (0.1 *M* phosphates) at 35°; or in 0.05 *M* Tris–HCl between 21 and 50°. (⊗). Over this temperature range, pH dropped from 7.7 to 7.0. Kinetics at 68° (⊗) were studied in pH 7 phosphate buffer. Points represent average readings from triplicate suspensions. Maximum variation, 0.01 OD unit.

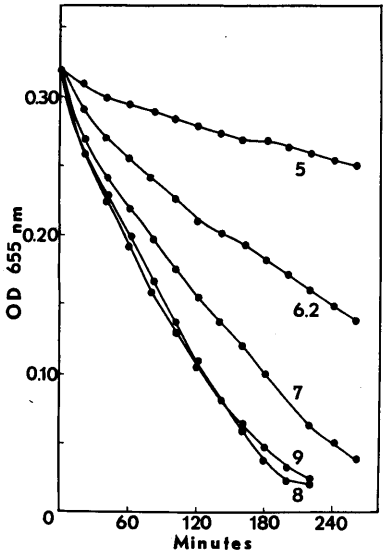

FIG. 3. Effect of pH on autolysin activity (Lot 1). Buffers at pH 5 and between 6.2 and 8 are described in Fig. 2. Tris–HCl (0.05 *M*) was used at pH 9. Temperature 35°. Points represent average readings from triplicate determinations. Maximum variation, 0.01 OD unit.

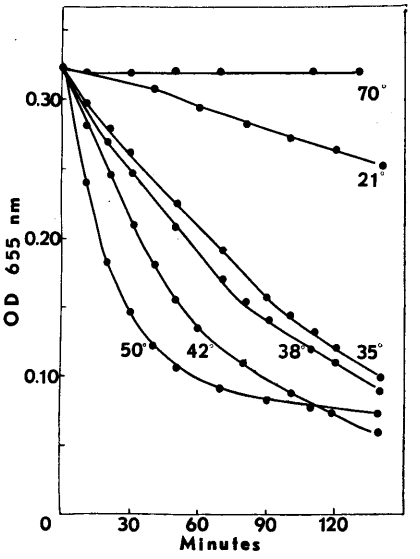

FIG. 4. Effect of temperature on autolysin activity (Lot 2). Incubation at indicated temperatures at pH 7 (0.1 *M* phosphate).

either possibility. More than one rate was also noted during wall dissolution and during the action of free autolysin on wall substrate.

Inactivation of free autolysin by temperatures potentiating wall lysis may be attributed to "structuring" of enzyme *in situ*. The similarity between pH optima for cell lysis and autolysin activity as opposed to wall dissolution, is not so simply explained. The potentiating effect of Tris–HCl on wall dissolution is absent in the free autolysin system. Further study of these discrepancies is in progress.

Summary. Cells and isolated walls of *Listeria monocytogenes* Strain 5 were labile, and autolysin was released from walls during dissolution. Cells, walls, and autolysin were similarly, but not identically, affected by pH and temperature.

1. Tyrrell, E. A., Trachtenberg, L., and Rajam, P. C., Proc. Soc. Exp. Biol. Med. **141**, 681 (1972).

2. Tinelli, R., Bull. Soc. Chim. Biol. **51**, 283 (1969).

3. Tyrrell, E. A., J. Bacteriol. **113**, 1046 (1973).

4. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

Received Mar. 29, 1973. P.S.E.B.M., 1973, Vol. 143.