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Autolysis in *Listeria monocytogenes:* Effect of Inhibitors of Cell Wall and Protein Synthesis (37969)

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Current theories of bacterial cell-wall maintenance ascribe a significant role to wall degradative enzymes (autolysins) which are presumed to act in balance with enzymes engaged in wall synthesis. Thus arrest of such synthesis or of insertion of wall subunits should result in imbalance possibly terminating in wall degradation and eventual cell lysis. In practical terms, several antibiotics that interfere with wall synthesis appear to exert bactericidal effects indirectly, due to the participation of wall autolysins (1, 2). Agents inhibiting protein synthesis, however, may be expected to restrict synthesis of both synthetic and degradative enzymes. Indeed, chloramphenicol (CMP) and tetracycline have been shown to inhibit lysis in the model *Streptococcus fecalis* autolytic system (3).

This report describes the effects of inhibitors of both wall and general protein synthesis on an autolytic strain of *Listeria monocytogenes.*

Materials and Methods. Brain–heart infusion broth (BHI, Baltimore Biological Laboratories) was used as the growth medium. Crystalline CMP and benzylpenicillin (1650 U/mg) were from Sigma Chemical Company. Vancomycin hydrochloride (1000 μg/mg) and tetracycline hydrochloride (980 μg/mg) were kindly donated by Eli Lilly Company and Pfizer Inc., respectively. Inhibitors were Millipore filtered before use.

Growth conditions. The derivation of *L. monocytogenes* Strain 5 has been described (4). Replicate 50-ml units of BHI were inoculated and aerated (4) at 35°. Growth and autolysis were followed by turbidimetry (4, 5) in the presence or absence of appropriate inhibitors. Untreated controls entered log phase at approximately 4 hr post-inoculation (PI) commenced deceleration at about 7 hr, and reached peak growth between 9 and 10 hr PI.

Inhibitors. CMP (100 μg/ml final concentration) was aseptically added to separate growing cultures at 5, 6, 7 and 8 hr PI, or (25 μg/ml) at 7 hr. Tetracycline, penicillin, or vancomycin (25, 5, and 25 μg/ml, respectively) were added at 7 hr PI. Triplicate cultures were used in each case and accompanied by triplicate untreated controls. No significant change in turbidity or pH was noted immediately after additions.

Results. As previously reported (4, 5), Strain 5 entered an autolytic curve, characterized by sequential rate changes, immediately after growth peak. The terminal linear rate began between 30 and 32 hr PI.

At 100 μg/ml final concentration, CMP added during early or late log phase (5 and 6 hr, respectively) or deceleration phase (7 hr) arrested multiplication. Time to arrest was maximum in early log phase and decreased with increasing culture age. Turbidity loss followed arrest in all cases, indicating premature onset of the autolytic phase (Fig. 1).

CMP (25 μg/ml) added at 7 hr PI also arrested multiplication, albeit more slowly. Arrest was followed by premature autolysis. Similar results were obtained with tetracycline, vancomycin, and penicillin (Fig. 2).

Addition of all these inhibitors, particularly penicillin and vancomycin, increased initial rates of turbidity loss without modifying time of onset of the terminal lytic

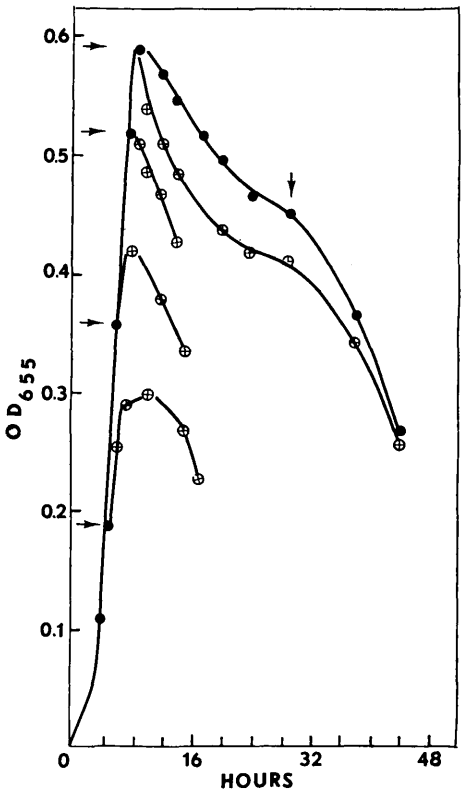

FIG. 1. Effect of CMP on growth and lysis of Strain 5. CMP was added to separate cultures at 5, 6, 7, or 8 hr postinoculation (horizontal arrows). Points are average readings from triplicate determinations. Maximum variation, 0.02 OD units. Vertical arrow denotes onset of terminal autolytic rate. CMP final concentration, 100 μg/ml. Untreated controls, solid circles. CMP cultures, open crossed circles.

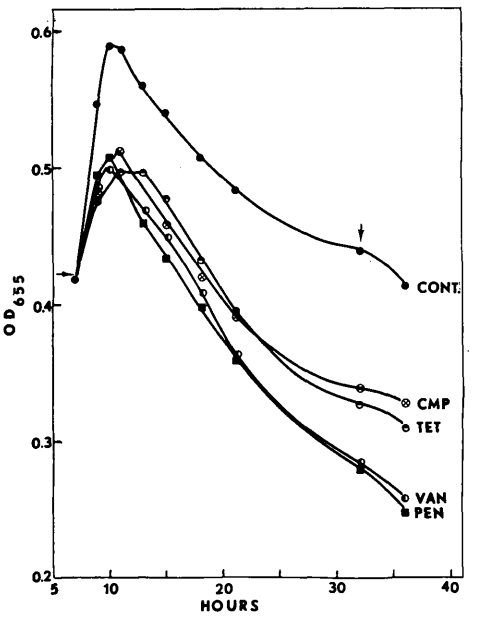

FIG. 2. Effect of inhibitors of wall or protein synthesis. CMP, tetracycline, vancomycin (final concentrations 25 μg/ml), and penicillin (5 μg/ml) were added 7 hr postinoculation (horizontal arrow). Points are average readings from triplicate determinations. Maximum variation, 0.03 OD units. Vertical arrow denotes onset of terminal autolytic rate. CONT., untreated controls. Abbreviations indicate cultures with chloramphenicol, tetracycline, vancomycin, or penicillin.

phase. This was also evident when CMP was added shortly before peak growth (Fig. 1).

Discussion. A distinction may be made between intrinsic autolytic capacity and conditions permitting expression of such capacity. Arrest of multiplication by CMP at several points on the growth curve was followed by turbidity loss, suggesting that cells of this strain were intrinsically autolytic under the conditions used. The fact that autolysis followed arrest by agents inhibiting either wall or general protein synthesis is compatible with unilateral action of the normal complement of wall autolysins, under conditions where wall synthesis was blocked by either direct or indirect routes. In certain microbial systems, autolysis mediated by inhibitors of wall synthesis is negated by prior arrest of protein synthesis (7). This is predictably not the case in the present system.

These results confirm the trend of prior findings with inhibitors of wall synthesis (1, 2) and extend them to an additional microbial genus. Unlike autolytic *Streptococcus fecalis* system (3), interference with general protein synthesis and negation of autolysis are not conjoint phenomena in this strain of *Listeria.* Since selective sparing of autolysin synthesis is unlikely, we suggest that preformed autolysins enjoy a significant *effective* half-life in the absence of synthesis *de novo.* This may be due to low metabolic turnover rates, the presence of a large autolysin pool, or possibly to activa-

tion of a pro-enzyme pool (8). A transport system for preformed autolysin (cytoplasm to wall) which is independent of protein synthesis is not excluded.

Though the terminal rate of turbidity loss is known to reflect protoplast formation (4), mechanisms underlying rate changes prior to this time have not been elucidated. Presumably they include the action of more than one autolysin and/or degradation of distant wall substrates (6). Thus the significance of increased initial rates following arrest by the inhibitors is not clear, particularly since time of onset of the terminal rate was unchanged. While it may be postulated that inhibitors of protein synthesis arrest synthesis of a putative short-lived inhibitor of autolysin activity, a comparable explanation cannot be easily envisioned for inhibitors of wall synthesis.

Summary. Premature autolysis was induced in a strain of *Listeria monocytogenes* when multiplication was arrested by inhibitors of either wall or general protein synthesis.

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