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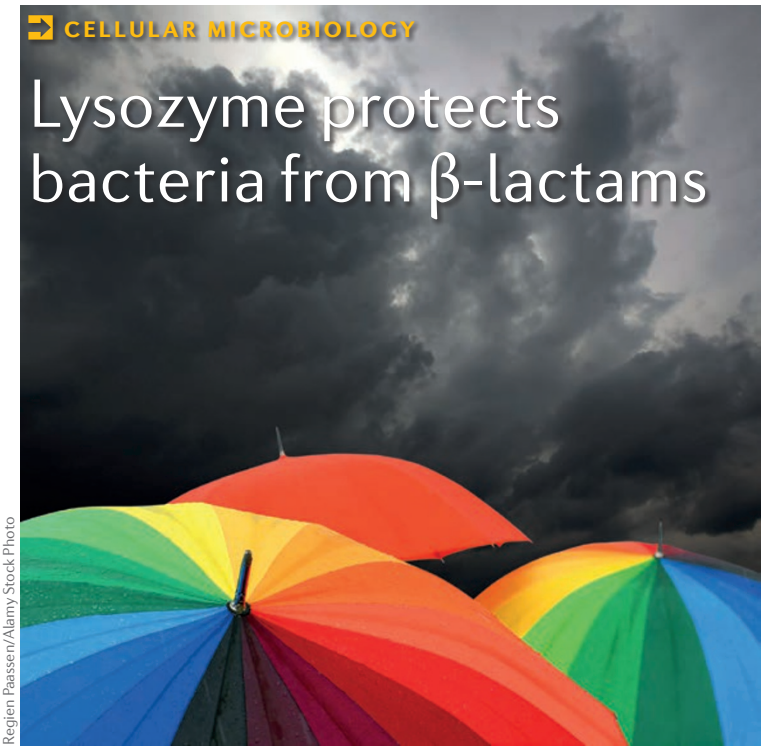
CELLULAR MICROBIOLOGY

Lysozyme protects bacteria from β-lactams



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β-Lactams target penicillin-binding proteins (PBPs), a group of enzymes that are involved in cross-linking peptidoglycan in the bacterial cell wall and that cause cell lysis under standard laboratory culture conditions, which are mainly hypotonic. Under physiological conditions, lysis of Gram-positive bacteria is suppressed in the presence of β-lactams, and cell growth can switch to a cell wall-deficient state called the 'L-form', albeit inefficiently. L-form bacteria are resistant to β-lactams and many antibiotics that target cell wall biogenesis; however, their potential role in recalcitrant and persistent infections is unknown. Moreover, the molecular basis for L-form switching in the host is unclear. Now, a recent study reports that efficient L-form switching is promoted by host lysozyme and potentially other innate immune effectors.

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Previous studies observed that inhibition of peptidoglycan precursor synthesis promotes the emergence and growth of L-form *Bacillus subtilis*; however, when penicillin was added to a strain in which precursor synthesis was repressed, L-form growth was significantly reduced. Previous work also found that efficient L-form cell formation was promoted by a mutation in a cell wall homeostasis regulator that the authors hypothesized increased autolytic activity in the cell wall, resulting in increased hydrolysis of cell wall peptidoglycan. In this study, depletion of two essential endopeptidases, LytE and CwlO, inhibited L-form growth, which was rescued through the addition of exogenous lysozyme, consistent with hydrolytic activity being essential for L-form growth. The authors hypothesized that penicillin blocked the action of cell wall hydrolases, resulting in the inefficient emergence of L-form bacteria. To test this, they cultured *B. subtilis* in the presence of penicillin and lysozyme and found that L-form growth occurred efficiently only when lysozyme was added, confirming that hydrolytic activity is required for the emergence of L-form bacteria. Using deletion mutagenesis, the authors found that class A PBPs are required for autolytic activity, and thus they concluded that although penicillin inhibits class A PBPs, this effect can be overcome by lysozyme.

As lysozyme is an important innate immune effector and there have been reports that exposure of bacteria to phagocytes induces L-form growth in vivo, the authors investigated L-form formation in *Galleria mellonella* (a simple insect model that possesses an innate immune system) and in mammalian macrophage cell culture. After challenging *G. mellonella* larvae with *B. subtilis* and *Staphylococcus aureus*, they observed L-form cells in association with larval phagocytes that could undergo L-form growth in the presence of penicillin. They also observed L-form cells escaping from mouse macrophages that were challenged with *B. subtilis* and subsequently cultured on penicillin-containing agar plates. Also, challenge of mouse macrophages with *S. aureus* in cell culture that contained penicillin led to the formation of L-form bacteria, which could not be isolated from a control culture incubated without macrophages. Moreover, treatment of *B. subtilis* with cellular extracts from macrophages and a cell type that does not produce lysozyme confirmed that the L-form switch is driven by host hydrolytic activity.

Altogether, these results show that lysozyme and potentially other lytic enzymes produced by host phagocytes protect bacteria from β-lactam-mediated killing, which the authors propose is through promoting the emergence of L-form bacteria that could act as persister cells in the host environment.

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ORIGINAL ARTICLE Kawai, Y., Mickiewicz, K. & Errington, J. Lysozyme counteracts β-lactam antibiotics by promoting the emergence of L-form bacteria. *Cell* **172**, 1–12 (2018)