

Research Article

Periplasmic lysozyme inhibitor contributes to lysozyme resistance in *Escherichia coli*

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Abstract. The product of the *Escherichia coli* ORFan gene *ykfE* was recently shown to be a strong inhibitor of C-type lysozyme in vitro. The gene was correspondingly renamed *ivy* (inhibitor of vertebrate lysozyme), but its biological function in *E. coli* remains unknown. In this work, we investigated the role of Ivy in the resistance of *E. coli* to the bactericidal effect of lysozyme in the presence of outer-membrane-permeabilizing treatments. Both in the presence of lactoferrin (3.0 mg/ml) and under high hydrostatic pressure (250 MPa), the lysozyme resis-

tance of *E. coli* MG1655 was decreased by knock-out of Ivy, and increased by overexpression of Ivy. However, knock-out of Ivy did not increase the lysozyme sensitivity of an *E. coli* MG1655 mutant previously described to be resistant to lysozyme under high pressure. These results indicate that Ivy is one of several factors that affect lysozyme resistance in *E. coli*, and suggest a possible function for Ivy as a host interaction factor in commensal and pathogenic *E. coli*.

Key words. Lysozyme; lysozyme inhibitor; *Escherichia coli*; defense mechanism; Ivy.

Lysozyme is the common name of a group of enzymes that can hydrolyze the bacterial cell wall polymer peptidoglycan by virtue of an N-acetylmuramoylhydrolase activity (E.C. 3.2.1.17). Lysozymes are commonly found in the major taxa of prokaryotes and eukaryotes, including the bacteria themselves, and also in bacteriophages, but they may have different roles in different organisms. For example, animal and plant lysozymes are important players in the defense against bacterial invaders [1–5], phage lysozymes play a role in phage penetration into, and/or release from the host cell [6, 7], while some bacterial lysozymes, called autolysins, allow controlled hydrolysis of the cell wall at sites of cell growth or cell division [8, 9]. The widespread natural occurrence of lysozyme, even in the human body, and its specificity against bacteria (peptidoglycan occurs exclusively but almost universally

in the phylum of the Bacteria), has fueled the development of applications of this enzyme as an antibacterial agent in foods, pharmaceuticals and cosmetics, and even for treating bacterial infections [10, 11]. One of the major limitations of lysozyme in this type of application is its poor efficiency against some bacteria. In particular, Gram-negative bacteria are generally insensitive to lysozyme because their peptidoglycan is protected by the surrounding outer membrane layer. However, two different approaches have proven useful to improve the efficiency of lysozyme against Gram-negative bacteria [12]. First, lysozyme can be structurally modified such that it can cross the outer membrane, probably by a mechanism related to self-promoted uptake [13, 14]. This has been accomplished by conjugation with a variety of molecules including fatty acids [15, 16], and also by extension of the polypeptide with a stretch of hydrophobic amino acids using a recombinant genetic approach [17]. Alternatively,

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