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A tangle of poly-phosphate in *Campylobacter*

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The intracellular level of inorganic polyphosphate (poly-P) is closely associated with stress responses, particularly stringent response (a regulatory mechanism in response to nutrient limitation), substantially impacting a variety of virulence-related phenotypes in pathogenic bacteria.[1] *Campylobacter jejuni* is considered as a leading foodborne pathogen worldwide;[2] however, the molecular mechanisms and regulation of the stress response and virulence of this pathogenic bacterium are still not completely understood. *C. jejuni* possesses several enzymes that are involved in the synthesis and degradation of poly-P and (p)ppGpp.[3,4] The intracellular levels of the alarmone (p)ppGpp, which mediates a stringent response, are controlled mainly by RelA and SpoT and significantly influence bacterial adaptation to environmental stress and pathogenicity.[5] The synthesis and degradation of poly-P directly affect (p)ppGpp levels and consequently stringent response. In most Gram-negative bacteria, both RelA and SpoT proteins are usually involved in the maintenance of the levels of (p)ppGpp; however, the stringent response of *C. jejuni* is governed by SpoT.[6] The expression of *spoT* is upregulated during *C. jejuni* infection of human intestinal epithelial cells, and its mutation renders *C. jejuni* defective in stationary phase survival, rifampicin resistance, and invasion and intracellular survival.[6] Consistently, a mutation of *ppk1*, whose protein product (PPK1, a polyphosphate kinase) is responsible for the synthesis of poly-P,[1,3] reduces *C. jejuni* survival within human epithelial cells and under stress conditions (e.g., oxidative stress and nutritional starvation).[3] Compared with PPK1, PPK2 has an opposite function on poly-P; whereas PPK1 synthesizes poly-P by using ATP, PPK2 degrades poly-P to convert GDP to GTP.[1,4] Like a *ppk1* mutant, a *ppk2* mutation also significantly affects *C. jejuni*'s capability of surviving under aerobic, osmotic and nutrient stresses.[4] Regarding biofilm formation, both *ppk1* and *ppk2* mutants exhibited augmented formation of biofilms,[3,4] despite their completely opposite functions on poly-P. In addition to PPKs, the exopolyphosphatases/guanosine pentaphosphate (pppGpp) phosphohydrolase (PPX/GPPA) also control the levels of poly-P and (p)ppGpp.[1] Malde et al. identified and characterized two PPX/GPPAs (PPX1 and PPX2) in *C. jejuni*, demonstrating that PPXs affect the levels of poly-P and ppGpp and contribute to the virulence of *C. jejuni*.[7] Mutations of *ppx1* and *ppx2* influenced various virulence-related phenotypes of *C. jejuni*, such as biofilms, motility, and invasion and intracellular survival.[7] These findings are consistent with the previous reports on other poly-P- and (p)ppGpp-associated genes in *C. jejuni*, such as *spoT*, *ppk1*, and *ppk2*; however, it is not straightforward to correlate some phenotypic changes in the mutants just based on the enzymatic activities on poly-P. For example, *ppk1* and *ppk2* mutations result in substantial reductions in the survival of *C. jejuni* under osmotic stress;[3,4] however, a single mutation of either *ppx1* or *ppx2* did not affect *C. jejuni* survival under osmotic stress and only a double mutation of *ppx1* and *ppx2* caused marginal reduction in bacterial counting under osmotic stress conditions.[7] Mutations of *ppk1*, *ppk2*, and *spoT* increased biofilm formation in *C. jejuni*,[3,4,6] while a single mutation of *ppx1* and *ppx2* did not affect biofilm formation and only a double mutation of *ppx1* and *ppx2* rather reduced biofilm formation.[7] A *ppk1* mutant demonstrates more substantial defects in intracellular survival than *ppx* mutants,[3] although the experimental settings in the studies are not exactly the same. Another difference between *ppk* mutants and *ppx* mutants can be found in motility. A *ppk1* mutation did not change motility,[3] whereas *ppx* mutations significantly reduced the motility of *C. jejuni*.[7] In addition, the *spoT* mutation substantially impairs *C. jejuni* resistance to rifampicin,[6] and a *ppk2* mutant exhibits increased susceptibility to antibiotics, such as erythromycin and tetracycline.[4] However, *ppx* mutations did not change the resistance to rifampicin, and the authors speculated that ppGpp levels in the *ppx* mutants would not be significant enough to affect rifampicin sensitivity.[7] Overall, comparison of the phonotype changes between *ppx* mutants and *ppk* mutants and the *spoT* mutant suggests that PPXs would be a complementary mechanism related to poly-P and stringent response. Based on the extent of phenotype changes (e.g., osmotic stress resistance and intracellular survival), PPKs appear to be the major mechanism affecting the virulence of *C. jejuni* through poly-P. As discussed above, some phenotype changes (e.g., motility and biofilms) in *spoT*, *ppk*, and *ppx* mutants cannot be explained simply based on their role in poly-P and (p)ppGpp, suggesting PPXs would be associated with the modulation of regulatory mechanisms other than stringent response. The study improved our understanding of the pathophysiological role of PPXs, but further studies appear to be needed to clarify the role of poly-P in the pathogenicity of *C. jejuni*.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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