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Molecular characterization of the type VII secretion system in *Bacillus* species

(バシルス属細菌 VII 型分泌装置の分子機能解析)

Bacillus species are a diverse group of Gram-positive bacteria found ubiquitously in nature. Among them of public health importance are *B. cereus* and *B. anthracis*, which are genetically closely related but possess distinct pathogenic profiles. *B. cereus* is an opportunistic pathogen commonly associated with foodborne illnesses with the potential for severe infection in immune-compromised hosts. In contrast, *B. anthracis*, the causative agent of anthrax, produces lethal toxins and a protective capsule, enabling severe systemic infections. Both species exhibit environmental resilience through spore formation, facilitating their survival and transmission. One of the significant virulence mechanisms Gram-positive bacteria possess is the type VII secretion system (T7SS), which plays a critical role in bacterial survival, pathogenesis, and host immune modulation by secreting effector proteins. The most common T7SS effectors are the early secreted antigenic target type A (EsxA) and B (EsxB), which possess a conserved Trp-Xaa-Gly (WXG) central sequence motif. However, the T7SS in *B. cereus* was previously uncharacterized.

Utilizing the *S. aureus* USA300 genome as a reference, whose T7SS is well-established, the T7SS core machinery genes were identified in the *B. cereus* ATCC14579. The T7SS locus comprised *esxA* (effector) and core machinery genes (*esaA*, *essA*, *esaB*, *essB* and *essC*). Further, liquid chromatograph-tandem mass spectrometry of culture supernatant EsxA1 and EsxA2 were found in the secretome of the wild type *B. cereus* ATCC14579 and none in its isogenic Δ *essC* strain. EsxA1 and EsxA2 possessed a 94% sequence identity. Comparative analysis found that the T7SS structural and effector genes were conserved

among *B. cereus* strains. However, *B. cereus* hospital isolates possessed additional virulence genes (*LXG* and *tigR*) downstream of *essC*, while environmental isolates did not, implying that T7SS variation may correlate with virulence. The T7SS-related genes in *B. anthracis* were not typically arranged, possessing *esxB* and five WXG100-like encoding genes scattered across the genome, including on the pXO1 plasmid. *B. anthracis* encodes EsaB and EssC typical T7SS machinery and SseB, a well-known T3SS translocon essential for virulence and pathogenicity in *Salmonella*.

To investigate the housekeeping roles of the T7SS, markerless gene deletions of *esxA1*, *esxA2*, *esxA1* and *esxA2*, *tigR* and *essC* were made in *B. cereus* ATCC14579. These deletions did not alter their morphology and growth rates. However, the $\Delta esxA1\Delta esxA2$ mutant significantly lost its ability to form mature spores efficiently. The $\Delta esxA1\Delta esxA2$ mutant had a significantly lower viable spore count, a reduction of almost 30% compared to the wild type and other T7SS mutants. Complementation of $\Delta esxA1\Delta esxA2$ with either *esxA1* or *esxA2* reversed the sporulation deficiency to levels of the wild type. EsxA was predicted to impact sporulation via SpoIIIE, a DNA translocase that translocates about 70% of the chromosome into the endospore. SpoIIIE and EssC belong to the FtsK/SpoIIIE ATPase family and possess high homology (96%) in the ATPase domain that binds the WXG100 proteins. These findings indicate the intrinsic link between one of the virulence molecules, EsxA, and the fitness of *B. cereus*, showcasing its potential for antimicrobial targeting.