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Biochemical characterizations of a RelE/ParE superfamily toxin in Vibrio parahaemolyticus

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論 文 名　：Biochemical characterizations of a RelE/ParE superfamily toxin in *Vibrio parahaemolyticus*
（腸炎ビブリオ由来 RelE/ParE スーパーファミリートキシンの生化学的解析）

区　　分　：甲

論　文　内　容　の　要　旨

Toxin-antitoxin (TA) systems, being abundant in bacterial and archaeal genomes, are small genetic elements composed of two genes organized on an operon which encodes a stable toxin and a labile cognate antitoxin, respectively. Under unfavorable conditions, such as amino acid poverty and DNA damage, the antitoxin is degraded, leading to the toxin activation, and consequently, the toxin arrests cell growth by its cellular effects, such as ribosome-dependent mRNA cleavage (mRNA interferase) or DNA gyrase (Gyr) inhibition. Although, TA systems are proposed to function as effectors of dormancy and persistence, their biological functions are still under debate. *Vibrio parahaemolyticus*, a seafood enteropathogen in coastal countries, causes acute gastroenteritis in humans. A characteristic feature of *V. parahaemolyticus* is that it can become viable, but not culturable (VBNC), at a low temperature in a minimum medium, in which a possible role of TA systems has been suggested. Previously, we found that two genes, *vp1842*/*vp1843*, within a superintegron on the *V. parahaemolyticus* genome, have homology to those encoding the *Escherichia coli* TA proteins, DinJ/YafQ. However, the toxin Vp1843, unlike the *E. coli* homologue YafQ, has no mRNA interferase activity. In this study, to gain insight into biological properties of Vp1842/Vp1843, I investigated the inhibitory potency of Vp1843 toward *E. coli* Gyr, and also phenotypically analyzed the *E. coli* cells expressing *vp1843*.

E. coli YafQ belongs to the RelE/ParE superfamily, whose toxins fold into a similar structure with distinct biological activities; RelE toxins are mRNA interferases, while ParE toxins have inhibitory activity toward Gyr. Since Vp1843 exhibited no mRNA interferase activity, I first tested whether Vp1843 could have any inhibitory activity toward *E. coli* Gyr using ciprofloxacin as a control. The result showed that Vp1843 had no influence on DNA supercoiling and relaxation activities of Gyr, but rather converted supercoiled DNA to open-circular DNA with nicks *via* a DNA nicking endonuclease activity. We further found that the antitoxin Vp1842 could neutralize the nicking activity of Vp1843, indicating that the strong toxicity of Vp1843 in *E. coli* is attributable to DNA damage due its nicking activity. To my knowledge, Vp1843 is the first toxin with DNA nicking endonuclease activity among the RelE/ParE superfamily.

Next, I explored whether *vp1842*/*vp1843* is involved in induction into the VBNC state by preparing a mutant strain (Δ1842/1843), in which *vp1842/vp1843* in the *V. parahaemolyticus* genome were knocked out by homologous recombination using a suicide vector pYAK1.

Unfortunately, the Δ1842/1843 mutant thus prepared, like wild-type *V. parahaemolyticus*, entered into the VBNC state under stress conditions, indicating that *vp1842/vp1843* is not involved in the VBNC state in *V. parahaemolyticus*. To establish a physiological function of *vp1842*/*vp1843*, the *vp1843* gene was expressed in *E. coli*, and the *E. coli* chromosomal DNA content was measured in DAPI (4',6-Diamidino-2-phenylindole) stained cells by FACS (Fluorescence-activated cell sorting), and nucleoids and cell membranes were visualized by phase and fluoresce microscopy. As a result, expression of *vp1843* caused the chromosomal DNA degradation, while the membrane of the cell remained intact. Since it is known that an extreme SOS responses caused by severe DNA damage induced apoptosis-like death characterized by DNA degradation in *E. coli*, the *vp1843* expression nicks the *E. coli* chromosomal DNA, which results in severe DNA damage, leading to DNA degradation and the apoptosis-like death. Interestingly, *vp1842*/*vp1843* locates in the superintegron in the *V. parahaemolyticus* chromosome, in which a large number of genes encoding proteins with no known function locate. Taken together, the present results suggest that when the superintegron including *vp1842*/*vp1843* within the chromosome is lost or damaged, the activated Vp1843 nicks the chromosomal DNA, which results in severe DNA damage, leading to DNA degradation and the apoptosis-like death. Thus, I propose that the TA system *vp1842*/*vp1843* may play a role in maintenance of the superintegron in the *V. parahaemolyticus* chromosome.