Abstract of Thesis

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Title	Interaction between *Bordetella bronchiseptica* and *Acanthamoeba* as a transient host in the natural environment （自然環境での生育を想定した、気管支敗血症菌とアメーバの相互作用）

Abstract of Thesis

Bordetella bronchiseptica (*Bb*) is a Gram-negative bacterium, which causes respiratory diseases in pigs, dogs, cats, and occasionally humans. In addition to infecting hosts, *Bb* is considered to survive in the environment persistently, which may provide a source of infection; however, the environmental lifestyle of *Bb* is poorly understood. In general, environmental bacteria survive in a dormant state or proliferate with or without interacting with other living organisms, such as protozoa.

In this study, I explored the possible interaction between *Bb* and *Acanthamoeba castellanii* (*Ac*) as a representative of environmental protozoa. Unlike bacteria that serve as amoeba food sources, *Bb* phagocytosed by the amoeba resisted digestion and escaped to extracellular milieu through contractile vacuoles (CVs), intracellular compartments involved in osmoregulation. Furthermore, *Bb* survived and proliferated for at least 28 days of co-culture with the amoeba. The Bordetellae harbors the *Bordetella* virulence gene (BvgAS) two-component system, which controls the reversible phenotypic conversions between an avirulent phenotype known as Bvg^- phase and the virulent Bvg^+ phase. A mutant of *Bb* locked in the Bvg^- phase but not in the Bvg^+ phase was found to survive and proliferate in the co-culture with the amoeba. Microscopic analyses revealed that the Bvg^+ phase-locked mutant was more efficiently internalized and transported to the intracellular digestion pathway than the Bvg^- phase-locked mutant. After seven days of co-culture, the dead cells of the Bvg^+ phase-locked mutant were accumulated in giant food vacuoles (GFVs), which were previously reported as a specific compartment in the amoeba phago-endosomal pathway. These data suggest that *Bb* resists predation by *Ac* in a Bvg phase-dependent manner. By screening variants of the Bvg^+ phase-locked mutant in which Bvg^+-specific genes were deleted, I found that deletion mutants deficient in bacterial adhesion molecules, FhaB and fimbriae, survived in the co-culture with the amoeba similar to the wild-type strain, indicating that the adhesion molecules are targeted for predation by the amoeba.

The present study indicates that *Bb* survives from *Ac* predation by concealing FhaB and fimbriae, which are major bacterial adhesins contributing to the establishment of bacterial colonization in infection to mammalian hosts. *Ac* could be a transient host in the natural environment, which supports the propagation of the bacteria. This study also provides a new perspective for understanding the ecology of *Bb* during the lifecycle outside the mammalian hosts.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究において申請者は、気管支敗血症菌（*Bordetella bronchiseptica*）の環境生存戦略の一端を明らかにするため、同菌とアメーバの相互作用を解析している。気管支敗血症菌は百日咳の原因菌である百日咳菌と同属の呼吸器感染を起こす病原細菌だが、宿主外では生存できないとされる百日咳菌とは異なり、自然環境下でも生育可能と考えられていた。しかし、宿主外環境での気管支敗血症菌の生育様態やそれに関わる細菌学的特性は全く解析されていなかった。申請者は、環境アメーバとの共存下で本菌がアメーバ内で消化されることなく増殖し、また本菌がアメーバを殺滅することもないという共生関係を見出し、この共生関係の成立には、本菌の環境応答のひとつである相転移（表現型変化）に伴う、接着因子（線毛および繊維状赤血球凝集素）の発現遮断が重要であることを明らかにした。これらの一連の研究は、病原細菌の宿主外での生態の理解に新たな視点を与えるもので、本菌の感染伝播に対応するための重要な知見を提供していると評価できる。以上のことから本論文は学位授与に相応しいとの評価結果を得た。