

氏名	HAN MIN OHN
授与した学位	博士
専攻分野の名称	学術
学位授与番号	博甲第6252号
学位授与の日付	令和2年9月25日
学位授与の要件	医歯薬学総合研究科 薬科学専攻 (学位規則第4条第1項該当)
学位論文の題目	Interaction of <i>Escherichia coli</i> with opportunistic pathogens during biofilm formation (バイオフィーム形成における大腸菌と日和見病原菌の相互作用)
論文審査委員	教授 小野 敦 (主査) 教授 垣内 力 准教授 金 惠淑

学位論文内容の要旨

In the present study, I explored whether interaction of *Escherichia coli* with either *Vibrio vulnificus* or *Staphylococcus epidermidis* effected the biofilm formation of these bacteria or not.

In the first part of the study, I explored how *E. coli* ATCC 35218 or its culture supernatant interacts with *V. vulnificus* L-180 during its biofilm formation. *V. vulnificus* is a foodborne pathogen causing septicemia with high mortality rate while *E. coli* is a commensal bacterium commonly present in the gastrointestinal tract of mammals including humans. My study showed that, the amount of biofilm produced by *V. vulnificus* L-180 was reduced in the presence of *E. coli* ATCC 35218, although the growth of *V. vulnificus* L-180 remains unaffected. I also found that even a minute amount of *E. coli* ATCC 35218 culture supernatant could interfere with the biofilm formation of *V. vulnificus* L-180. *E. coli* ATCC culture supernatant could also reduce the amount of preformed *V. vulnificus* biofilm. In addition, I found that antibiofilm effect of *E. coli* ATCC 35218 culture supernatant against *V. vulnificus* L-180 did not get reduced even after heat treatment. These findings indicate that *E. coli* and its culture supernatant may be suitable to prevent the biofilm formation by *V. vulnificus*. On the other hand, *V. vulnificus* L-180 living cells could reduce the amount of preformed *E. coli* ATCC 35218 biofilm, but culture supernatant could not. This suggests that the cell-associated factors contribute towards reduction in the *E. coli* ATCC 35218 biofilm. Therefore, I speculate that ingestion of infectious dose of *V. vulnificus* might induce dislodging of the commensal bacteria from the intestine and thus can colonize to initiate the infection.

Second part of the study demonstrated that a commensal bacterium *E. coli* might prevent the biofilm formation of *Staphylococcus epidermidis*, a skin commensal bacterium which is also a nosocomial pathogen. Staphylococci, including *S. epidermidis*, are also regularly isolated from the GI tract of infants and small children. When co-cultured with *S. epidermidis* ATCC 35984, the cells of *E. coli* ATCC 35218 dominated in both culture fluid and biofilm. In addition, *E. coli* ATCC 35218 significantly incorporated into and grew in a niche preoccupied by *S. epidermidis* biofilm. However, *S. epidermidis* ATCC 35984 could not incorporate well into a niche preoccupied by *E. coli* ATCC 35218 biofilm. Although far greater amount was required and less efficient, the culture supernatant from *E. coli* ATCC 35218 also showed to reduce the amount of biofilm formed by *S. epidermidis* ATCC 35984 and the component(s) of the culture supernatant that exhibit antibiofilm activity were also found to be heat-stable. *E.*

coli culture supernatant, however, did not have any effect on preformed *S. epidermidis* biofilm. Two other *E. coli* strains (strain K12 and B) were also able to interfere with the formation of *S. epidermidis* ATCC 35984 biofilm. These findings suggest that, through inhibition of the biofilm development and growth, *E. coli* and its culture supernatant may take part in preventing colonization of *S. epidermidis* in the adult gastrointestinal tract. In addition, my findings also suggest that *E. coli* may also destabilize *S. epidermidis* colonizing the GI tract of infants and small children and may be useful in removing potentially pathogenic *S. epidermidis* colonizing the GI tract of infants and small children.

論文審査結果の要旨

審査結果に至った理由: 近年、腸内フローラの健康への役割が注目されており、その一つに病原菌からの生体防御が挙げられる。本研究では、腸管内共生菌である非病原性大腸菌と日和見菌であり食中毒原因菌となる *Vibrio vulnificus* および *Staphylococcus epidermidis* のバイオフィーム形成における相互作用について検討を行った結果、大腸菌 (*E. coli* ATCC 35218) およびその培養上清が、*V. vulnificus* L-180 や *S. epidermidis* ATCC 35984 のバイオフィーム形成を阻害すること、培養上清中の阻害因子は耐熱性であることを明らかとした。また、3種の系統の *E. coli* ATCC 35218, K-12, B で *S. epidermidis* ATCC 35984 のバイオフィーム形成へ及ぼす影響は同程度であることを明らかとした。これらの結果は腸管に共生する大腸菌の食中毒菌の腸管内でのコロニー形成からの防御機構を示していると考えられた。

審査委員会では、内容について詳細な議論を行い、これまでに報告された関連研究における既知見と本研究の新規知見が何であることを明確に示すことや、研究に用いた菌株の特性や選択理由などについて示すこと、また統計解析や写真の追加などを求めた。後日、修正版が提出され、審査委員会での指摘を踏まえた適切な追加・修正がなされていることを確認した。

以上より、本論文は、腸管内共生細菌である大腸菌およびその培養上清による食中毒菌 *V. vulnificus* や皮膚共生細菌で乳幼児食中毒の原因菌でもある *S. epidermidis* によるバイオフィーム形成を阻害する作用を確認し、それらの細菌による感染制御への応用が期待される成果を示すものであり、博士(薬学)の学位に値するものと判断した。