

## 最終試験の結果の要旨

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主査および副査の5名は、平成 28 年 02 月 10 日、学位申請者 SHAMMI FARIHA AFSANA 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。

質問 1) How does the bacteriocin work on the *S. aureus*?

(回答) Bacteriocin disrupts the cell membrane and forms the pore on the cell membrane, causing cell death.

質問 2) In slide 7, gene expressions of *sacA* and *orf46* were different. Why did such a phenomenon occur?

(回答) We hypothesis that immunity factors may express after bacteriocin export outside the bacteria to protect by themselves.

質問 3) What is two-component system?

(回答) This system is bacterial specific signal transduction system. It is consisted of at least two peptides, histidin kinase and response regulator. Histidin kinase detects the environmental stimuli and transfers the signal to cognate response regulator (RR) and finally activates-RR and regulates various gene expression.

質問 4) In Yamaguchi et al. paper, why was the result of direct assay against 209P different from your research?

(回答) Because conditions of temperature and cell volume of direct assay are different from Yamaguchi's method.

質問 5) Why was the result of susceptibility different between *orf46-47* and *orf48*?

(回答) Because *orf46-47* coding ABC transporter may be a major resistance factor. *orf48* encodes possible protein of ABC transporter. Normally ABC transporter is a major resistance factor.

質問 6) What is the difference between class I and class II bacteriocin?

(回答) Class I bacteriocins are also called lantibiotics. Lantibiotics contain an unusual amino acid, lanthionine, which is post-translationally modified to methyl lanthionine but class II bacteriocin is non-lanthionine bacteriocin and amino acid sequence is different from class I group.

質問 7) Is class I more important compared to class II?

(回答) Both bacteriocins are important. Just only structures and mode of action are different.

質問 8) Does the production ability of C55 bacteriocin change depend on the environmental change?

(回答) We don't have any evidence but it is supposed that environmental change may affect the bacteriocin-related gene expression, causing the change of bacteriocin production.

質問 9) In figure 4, why didn't you explain about OD?

(回答) I explained about OD in materials and method.

質問 10) Does this bacteriocin have bactericidal or bacteriostatic activity?

(回答) This bacteriocin has bactericidal activity against plasmid-negative *S. aureus* strains.

質問 11) Are ETA and ETD necessary for competition?

(回答) I don't think so. Because only *S. aureus* which has pETB plasmid has the ability to produce bacteriocin. ETB-producing *S. aureus* has pETB plasmid whereas ETA/ETD-producing *S. aureus* has no pETB plasmid, suggesting that they don't have ability to produce bacteriocin.

質問12) Does ETB-bacteriocin interfere with the Gram-negative bacteria?

(回答) I didn't check the effect against Gram-negative bacteria, but normally type I bacteriocin has little effects against Gram-negative bacteria. C55 may not have effects against Gram-negative bacteria.

質問13) In figure 6A you used Student's t-test, but in figure 6c used Dunnette's test for statistical analysis, what is the difference?

(回答) I am sorry it's my mistake. In co-culture assay, we used Dunnette's test. We will revise the manuscript.

質問 14) Does the ETB-positive *S. aureus* commonly show higher virulence than the ETB-negative or ETA and/or ETD?

(回答) The gene encoding ETB is located in the plasmid, so ETB-negative *S. aureus* doesn't produce toxin. ETA-producing *S. aureus* has similar virulence as ETB-producing *S. aureus* and produces different type of bacteriocin.

質問 15) Can *S. epidermidis* produce any C55-like lantibiotics as well as C55-producing *S. aureus* immunity factor?

(回答) It was reported that one of *S. epidermidis* also produced lantibiotics (epidermin), different type of bacteriocin compared to C55 bacteriocin. Epidermin has one peptide component and C55 has two peptide components.

質問16) Are the gene expression level of *sacA* and that of *orf46* directly proportional to the peptide expression level of C55 and that of *orf46*, respectively?

(回答) I only checked the RNA expression level of bacteriocin synthesis gene (*sacA*) and immunity gene *orf46* during growth, but I didn't check the protein expression. We suppose that RNA expression level reflect peptide expression level.

質問17) You wrote "the synthesis gene transcripts increased 70-fold than the transcripts of the immunity factors." I would like to get your opinion.

(回答) I agree with you, the higher and lower value indicate the bactericidal and or bacteriostatic activity of the bacteriocin and also the lowest values during the early exponential growth phase are very important. Now I am not sure the efficiency of immunity factors is higher or not. At 9-hour incubation, I checked the viability and also CFUs. We found that almost all bacterial cells were viable. TY4 can grow well and also is not killed by own peptide during growth. But I am not sure because I did not check.

質問18) Can the findings obtained in this study be utilized to the pharmacological treatment of diseases caused by *S. aureus* or other bacteria? Could I get your opinion?

(回答) My hypothesis, bacteriocin interferes with other bacteria in the bacterial community. Because C55 bacteriocin exhibits killing activity against *S. aureus*. So, C55 producing *S. aureus* may inhibit pETB-negative *S. aureus* strains resulting in the spread of infection. If we use this purified bacteriocin (in future) to control bacterial infection, medical and dental science will be benefitted. In my opinion, it is very difficult to use clinically because C55 is produced by pathogenic bacteria.

質問19) What is the role of immunity factor?

(回答) Resistance against the bacteriocin produced by themselves.

質問 20) Which region is encoding ETB in *orf* map?

(回答) Bacteriocin encoding region is located on the other area in pETB-plasmid. In *orf* map I focused on only immunity factors but not ETB encoding gene.

質問 21) Why was the clearing zone of 209P bigger compared to that of MW2?

(回答) I didn't know the exact reason. I hypothesize that MW2 strain have some resistance factor against C55 bacteriocin.

質問 22) What is the role of ApsR?

(回答) ApsR regulates the membrane charge in *S. aureus*. ApsR-deficient mutants, which have increased negative-charge, are susceptible to cationic peptides.

以上の結果から、5名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士(歯学)の学位を与えるに足る資格を有するものと認定した。