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 29 年 2 月 9 日、学位申請者 Atik Ramadhani 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。

Question 1: Why did you use bacterium *Paracoccus pantotrophus*?
Answer : The Sox enzyme system in *P. pantotrophus* has been studied so far for sulfur oxidation.

Question 2: Why did you choose *P. pantotrophus* GB17, and is this strain available commercially?
Answer : Compared to other strains, GB17 has mainly been studied so far, and it is available commercially.

Question 3: Where can we find *P. pantotrophus*?
Answer : *P. pantotrophus* can be found in soil, water, mud, sewage, rocks, and sludge.

Question 4: Are there any bacteria that can catalyze taurine, which is one of sulfur-containing compounds?
Answer : As indicated, taurine may be a source of H_2S. As far as I know, bacteria of that nature have not been reported.

Question 5: Why did you use rSoxCD combination and why did only rSoxC react in Western Blot analysis?
Answer : We constructed a complex form of SoxC and SoxD, and 6×His was attached to rSoxC.

Question 6: Why can we observe 3 bands of rSoxZ in SDS-PAGE analysis?
Answer : rSoxZ was separated into three bands. We suggest that rSoxZ migrated at 22 kDa, while 43-kDa band was a dimer and 21-kDa band lacked some portion of rSoxZ.

Question 7: How did you identify rSox proteins because molecular masses of them were different from those predicted by nucleotide sequence?
Answer : We confirmed the identities of all *sox* genes of *P. pantotrophus* GB17 by BLAST nucleotide sequence analysis. Amino acid composition and predicted pI value of each rSox protein did not seem to affect the mobility of the proteins on SDS-PAGE gels. Several investigators have also shown the same discrepancy and the reason for it remains unclear.

Question 8: What is the reason why rSox enzymes did not degrade H_2S completely to 0?
Answer : In the assay mixture, H_2S is generated from NaHS constantly. We think rSox enzyme could degrade H_2S completely if the production of H_2S would stop.

Question 9: Can you smell H_2S in 5 ng/ml concentration in the present assay?
Answer : No, we could not detect it.

Question 10: Regarding the time-course experiment, why didn't you measure rSox enzymes activity at time 0, and did you measure the amount of H_2S between 1 and 2 hours?
Answer : At time 0, it was impossible to measure rSox enzymes activity because H_2S was not produced. And we did not measure the amount of H_2S between 1 and 2 hours.

Question 11: In the thermal stability study, why didn't you examine the effect of heat treatment at 37°C (body temperature)?
Answer : We thought the result would be almost similar to that obtained at room temperature.

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Question 12: Did you examine rSox enzymes activity below 25°C?
Answer : No, we did not determine it.

Question 13: Why are rSox enzymes heat-stable?
Answer : Thermophilic sulfur-oxidizing bacteria have been isolated from geothermal fields and there has been a report suggesting that Sox system originated in ancient thermophilic bacteria and evolved through extensive horizontal gene transfer.

Question 14: Regarding the experiment of pH effect, why did you use the ratio to express the H_2S-degrading activity?
Answer : Because the H_2S levels generated from NaHS were different among different pH conditions, we used the ratio.

Question 15: Regarding the experiment examining the effect of each rSox component, what was the ratio of each enzyme in the assay mixture?
Answer : Mixture with equimolecular amount of each enzyme was used according to the previous reports.

Question 16: Are there any proteins similar to Sox enzymes in mammals?
Answer : Sox pathway is only possessed in environmental bacteria.

Question 17: What are non-pathological causes of oral malodor?
Answer : Smoking, spicy foods or garlic, drugs, and drinking alcohol are included.

Question 18: Do you think dry mouth can induce oral malodor?
Answer : Dry mouth often causes an increase in plaque deposition on tooth and tongue surfaces, and decrease in salivary flow leads to the lack of antimicrobial and cleaning activity of the saliva. We think those outcomes can increase oral malodor.

Question 19: How do oral bacteria attach to the surfaces in the oral cavity?
Answer : Several bacteria attach to acquired pellicle formed on the tooth surface via fimbriae and fibrils of them. Some bacteria attach to interspaces within tongue papillae.

Question 20: Final product of Sox reaction (H_2SO_4) is acidic. What do you think about the presence of this component in the oral cavity?
Answer : It might cause adverse effects on oral tissues. On the other hand, saliva has buffering capacity to neutralize acids and several oral bacteria produce neutralizing factors (alkaline proteins). Further studies are necessary to clarify the effect of final product H_2SO_4 in the oral cavity.

Question 21: How does H_2S increase the permeability of oral mucosa?
Answer : Once synthesized intracellularly, proteoglycans and glycoproteins are secreted and are held in an aggregated state through disulfide bridges in the extracellular matrix. H_2S may induce de-aggregation of proteoglycans by cleaving disulfide bonds and induce an increase in the permeability of oral mucosa.

Question 22: How about the toxicity of this enzyme?
Answer : We found no articles reporting toxicity of Sox enzymes on human body. However, further studies are necessary to clarify it and stability of rSox enzymes against proteases present in saliva is also required.

Question 23: How will you apply rSox enzymes clinically in the future?
Answer : There are possible application forms including mouth rinses or mouth fresheners.

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。