■アップデートシンポジウム 10（US10）

日時：9 月 6 日（土）9:00 ～ 10:30
会場：G 会場（会議場 3 階　33 会議室）
座長：鷲尾 純平（東北大　院歯　口腔生化）
　　佐藤 拓一（新潟大　院保　臨床化学）
　　大島 朋子（鶴大　歯　微生物）
　　泉福 英信（日大　松戸歯　感染免疫）
　　永野 恵司（北医療大　歯　微生物）
　　眞島 いづみ（奥羽大　歯　口腔病態解析制御）

「The Current Reports on Oral/Systemic Microbiome and Microbiota by Promising Challengers」

9:00 ～ 9:05
オーバービュー　座長

09:05 ～ 09:21

US10-1　「Microbiota profiling of the remaining bottled black tea and coffee beverages」

Miho Kawachi[1]、Haruna Sato[1]、Anna Wakui[1,3]、Yuki Kato[1]、Hiroto Sano[1,2]、Yuki Abiko[4]、Jumpei Washio[4]、Takuichi Sato[1]
([1] Div of Clin Chem, Niigata Univ Grad Sch Health Sci、[2] Nippon Dent Univ at Niigata、[3] Dept of Med Technol, Niigata Univ Health Welfare、[4] Div of Oral Ecol Biochem, Tohoku Univ Grad Sch Dent)

Resting saliva was collected and inoculated into the plastic bottles of black tea/coffee with/without sugars/milk; and then the survival of oral bacteria and their characteristics were examined after storage at 37℃ for 24 h. Resting saliva was collected from 14 healthy subjects, and then inoculated as 1.8×10^3 CFU/mL into the plastic bottles of black tea and coffee. After 1-day, the samples in the bottles were inoculated onto blood agar plates, incubated anaerobically at 37℃ for 7 days, and bacterial species were identified by 16S rRNA gene sequencing. The amounts of bacteria of the black tea (without sugars/milk, 14 cases) were only 10^1 levels, while those (with sugars; 3.9%, 7 cases) were from 10^1-10^3 levels. In contrast, the bacterial amounts of the black tea (with only milk, 4 cases) were $(3.3\pm0.7)\times10^7$. Furthermore, the bacterial amounts of the black tea (with sugars and milk, 4 cases) were $(4.7\pm2.6)\times10^7$. The bacterial components will be discussed in the presentation. The adding of sugars to the black tea gives little impact, and the black tea/coffee beverages without milk may possibly be preserved for a longer period.

09:21 ～ 09:37

US10-2 「Effects of eDNA and insoluble glucan on the biofilm formation of *Staphylococcus aureus*」

Toshiki Uematsu[1]、Hidenobu Senpuku[2]
([1] 5th year under graduate student, Nihon Univ Sch Dent at Matsudo、[2] Dept Microbiol Immunol, Nihon Univ Sch Dent at Matsudo)

Oral biofilm formation (BF) was strongly associated with insoluble glucan synthesized by glucosyltransferase from *Streptococcus mutans*. and *Staphylococcus aureus*, which has also been detected in the oral biofilm, has been reported as an opportunistic pathogen. In this study, relationships of BFs between *S. aureus* and *S. mutans* were investigated. The effects of regulation signals to synthesize various polysaccharides and to lead quorum sensing (QS) in *S. mutans* were investigated on the BF of *S. aureus*. Sonic extracts (SE) from mutants to glucosyltransferase and QS genes, and other genes were added with or without DNase I into *S. aureus* cowan I on microtiter plates in tryptic soy broth with 0.25% sucrose. After incubation, the biofilm cells were stained with 0.25% safranin and assessed by the absorbance at 492 nm. SE from mutants of the QS-related genes could not induce BF of *S. aureus*. The biofilm was induced by SE from mutants of the glucan-related genes, *gtfB*, *gtfBC* and *gbpC*, but inhibited by DNase I. These results suggested that the QS-components and extracellular DNA (eDNA) might be important factors in complex biofilm formation of *S. mutans* and *S. aureus*.

09:37 ～ 09:53

US10-3 「Acidogenicity and fluoride resistance of oral *Candida* species under different environmental conditions.」

Haneen Raafat Fathi Mousa[1,2]、Yuki Abiko[1]、Jumpei Washio[1]、Satoko Sato[1]、Nobuhiro Takahashi[1]
([1] Div Oral Ecol Biochem, Tohoku Univ Grad Sch Dent、[2] Ped Dent and Dent Public Health, Faculty Dent, Ain Shams Univ, Egypt)

Candida species (*C.* spp.) have been associated with dental caries. However, the acid production potential, particularly under anaerobic conditions such as deep carious lesions, and the sensitivity to fluoride have not been studied well. Therefore, this study aimed to evaluate the cariogenic potential of 5 strains of C. spp., and the effect of fluoride on them.

Under anaerobic conditions, growth of all *C.* spp. was inefficient, but they could survive, remain metabolically active, and produce acids. Furthermore, all except C. glabrata were extremely resistant to high concentrations of fluoride (80 mM). In contrast, enolase extracted from *Candidal* cells was inhibited by lower concentrations of fluoride, the 50% inhibitory concentration (IC_{50}) being as low as 0.19-0.34 mM. These results suggest that *C.* spp. possess a mechanism to maintain low intracellular fluoride concentration or to render it ineffective.

In conclusion, *C.* spp. have cariogenic potential even under anaerobic conditions, and were extremely resistant to fluoride. It may be important for the prevention of dental caries to consider novel approaches other than fluoride to combat the cariogenic potential of fungal species.

09:54 ～ 10:09

US10-4 「Regulatory mechanisms of outer membrane permeability in *Pseudomonas aeruginosa* : implications for antibiotic resistance」

Chen-Hsuan Chiu[1]、Rei Kobayashi[1]、Keiji Nagano[1]
([1] Div Microbiol, Dept Oral Biol, Health Sci Univ Hokkaido Sch Dent)

P. aeruginosa exhibits intrinsic resistance to many antibacterial agents, largely due to low outer membrane permeability. Enhancing this permeability could improve antibiotic efficacy. This study aimed to identify genes involved in this trait. A *P. aeruginosa* PAO1 strain was engineered to metabolize raffinose (Raf) and stachyose (Sta), then subjected to transposon-mediated random mutagenesis and chemostat culture in minimal medium with Raf as the sole carbon source. Selected mutants showed comparable growth to the parent strain in glucose but significantly faster growth in Raf and Sta. MIC assays revealed a 2- to 4-fold increase in sensitivity to ten antibacterial agents, indicating enhanced permeability to large molecules. DNA sequencing identified mutations in the morA gene in all mutants; *morA* encodes a known cyclic di-GMP receptor. No significant changes were observed in OprF porin expression, which plays a pivotal role in permeability to high-molecular-weight substances. Further research is needed to clarify the mechanisms, including potential structural alterations in OprF.

10:09 ～ 10:30

US10-5 「Antifungal Activity of Probiotic Lactobacilli Culture Supernatant against *Candida albicans* -the Possibility of Suppression Factor Other than Organic Acids」

Shi Qiuyi[1]、Yukako Kojima[2]、Yoko Mukai[2]、Chikahiro Ohkubo[1]、Tomoko Ohshima[2]
([1] Dept Oral Rehabilitation Prosthodont, Tsurumi Univ Sch Dent Med、[2] Dept Oral Microbiol, Tsurumi Univ Sch Dent Med)

Objectives: To prevent the emergence of drug-resistant Candida strains, probiotics lactobacilli strains that exhibit potent antifungal activity has been proposed as an alternative therapy. However, lactobacilli are known to produce acidic product that may cause unwanted effect in oral cavity. In this study, we examined antifungal property of lactobacilli culture supernatant (LCS) against *Candida albicans* using transcriptomics to explore the mechanism.

Methods: The antifungal activities of LCS were examined against yeast and hyphal forms of *C. albicans*. The transcriptome analysis of *C. albicans* treated with LCS was carried out by mRNA sequencing, and qRT-PCR was performed to confirm the variations in gene expression.

Results and discussion: Three Lactobacillus strains which demonstrated highest antifungal activity were selected. The result of transcriptome analysis indicated that the specific genes expression of *C. albicans* were down-regulated by LCS, those profiles were different from lactic acid-treated *C. albicans*, implying the presence of other active ingredients. Further studies are required to demystify the mechanism of antifungal action exerted by the LCS.