I_Host-Microbe Interactions and Pathogenesis

I-1

Effective Principle to Release the Gray Mold Weapon

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Gray mold is an important necrotrophic fungal pathogen that causes huge economic losses in agriculture. Oxalate is a key weapon of gray mold. The principle of this study was to find a way to effectively release gray mold weapons and apply them to the disease control. We isolated non-antifungal oxalate-degrading bacteria (ODB) from the surfaces of oxalate-rich spinach and strawberries to evaluate their ability to control gray mold. A total of 36 bacteria: 27 bacteria isolated from spinach; and 9 bacteria isolated from strawberry grown on minimal media with oxalate as a sole carbon source were evaluated for oxalate-degrading activity. Two isolates exhibiting the highest oxalate degradation and non-antifungal activity were subjected to disease suppression assays. *Pseudomonas abietaniphila* ODB36, which exhibited significant plant protective ability, was finally selected for further investigation. Based on whole-genome information, the *p*seudomonad *o*xalate degrading (*podA*) gene, which encodes formyl-CoA transferase functioning oxalate degradation, was identified. The *podA*$^-$ mutant does not inhibit *Botrytis* infection and oxalate toxicity; the defects were recovered by *podA* complementation. Purified PodA-His converted oxalate to formate and eliminated oxalate toxicity. These results indicate that *P. abietaniphila* ODB36 PodA release effectively the oxalate weapon of gray mold.

Keywords : Gray mold, oxalate, *podA*

I-2

Exosomes Released from Shiga Toxin 2a-Treated Human Macrophages Modulate Inflammatory Responses and Induce Cell Death in Toxin Receptor Expressing Human Cells

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Shiga toxins (Stxs) produced by Stx-producing *Escherichia coli* are the primarily virulence factors of hemolytic uremic syndrome and CNS impairment. Although the precise mechanisms of toxin dissemination remain unclear, Stxs bind to extracellular vesicles (EV). Exosomes, a subset of EVs, may play a key role in Stx-mediated renal injury. To test this hypothesis, we isolated exosomes from monocyte-derived macrophages in the presence of Stx2a or Stx2 toxoids. Macrophage-like differentiated THP-1 cells treated with Stxs secreted Stx-associated exosomes (Stx-Exo) of 90-130 nm in diameter, which induced cytotoxicity in recipient cells in a toxin receptor globotriaosylceramide (Gb3)- dependent manner. Stx2-Exo engulfed by Gb3-positive cells were translocated to the endoplasmic reticulum in the human proximal tubule epithelial cell line HK-2. Stx2-Exo contained pro-inflammatory cytokine mRNAs and proteins, and induced more severe inflammation than purified Stx2a accompanied by greater death of target cells such as human renal or retinal pigment epithelial cells. Blockade of exosome biogenesis using the pharmacological inhibitor GW4869 reduced Stx2-Exo-mediated human renal cell death suggesting a new promising strategy for Stx-mediated diseases.

Keywords : Shiga toxin, exosome, Gb3

I-3

Two *Streptococcus S*pecies Show Antagonistic Effects against *Helicobacter pylori* and Their Genome Sequences Reveal Possible Mechanisms

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Gastric cancer is a buildup of abnormal cells that form a mass in part of the stomach. It can develop in any part of the stomach, and Japan and South Korea have high rates of incidence. More than 60% of gastric cancer cases are due to *Helicobacter pylori* infection. Upon the colonization of the bacterium on the stomach, cancer development is induced by major virulence factors such as the type IV secretion system encoded in the Cag pathogenicity island. Therefore, the removal of *H. pylori* is required to prevent carcinogenesis. To meet the demand, we screened our in-house gastric culture collection to identify bacteria antagonistic against *Helicobacter* that interfere with the infection of *H. pylori* or suppress its growth. We first established a culture collection, Bank of Gastric Microbiota, from gastric biopsy samples has been established, and over 900 bacterial isolates have been successfully recovered. Screening tests found two *Streptococcus* species showing antagonistic effects against *H. pylori*. In addition, further liquid co-culture assay implied that the antagonistic activities of two isolates function through a contact-independent mechanism. Subsequently, whole genome sequencing of two bacteria was performed to investigate the factors associated with antagonistic effects using a hybrid sequencing strategy that employed PacBio and Illumina NovaSeq 6000. Initial sequence analyses suggest that the antagonistic effects against *H. pylori* could be mediated by bacteriocins or secondary metabolites.

Keywords : Gastric cancer, *Helicobacter pylori*, whole genome sequencing

I-4

Establishment of a Gastric Culture Collection and Screening Microbes for Antagonistic Activity against *Helicobacter pylori*

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Gastric cancer is the third leading cause of death globally with higher incidences in Korea and Japan. *Helicobacter pylori* is considered as the most severe risk factor of gastric cancer, since over 60% gastric diseases are caused by *H. pylori* infection. It is gram-negative, spiral shaped, and acid-tolerant, and grows under the microaerophilic condition. Additionally, the Cag pathogenicity island and the type IV secretion system severely promote the gastric cancer development by disrupting host signaling system. Therefore, eradication of this bacterium in the stomach is prerequisite to prevent the cancer development. Despite of well-established treatment regimens, the emergence of antibiotic resistance and patient compliance hinder proper treatments. To overcome these issues, microbial-interaction mediated eradication is proposed for the removal of *H. pylori* in this study. To this end, the bacterial culture collection from stomach biopsies has been established, and over 900 isolates have been successfully collected followed by taxonomic identification. Subsequently, antagonistic tests against *H. pylori* have been performed by screening out 904 isolates. Antagonistic effects were observed from 12 isolates, and further co-culture assay confirmed the growth suppression effects from two *Streptococcus* species. The whole genome sequencing of two species has been progressed to look into factors that are responsible for the antagonistic effects. In conclusion, potent bacteria for the removal of *H. pylori* have been identified, and further safety issues and the antagonistic effects *in vivo* are warranted.

Keywords : Gastric cancer, *Helicobacter pylori*, antagonistic effects

I-5

Metabolites Profiling of *Caenorhabditis elegans* Infected by EHEC O157:H7 Using GC-MS

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Enterohemorrhagic *Escherichia coli (EHEC)* O157:H7 is a pathogen that causes serious human disease outbreaks through the consumption of contaminated animal-associated food or water. In this study, we employed nematode *Caenorhabditis elegans* as a host model system to elucidate specific host responses during EHEC O157:H7 infection using global metabolomic analysis by gas chromatography mass spectrometry (GC-MS). To compare the metabolic differences between control and EHEC O157:H7 group, principal component analysis (PCA) and independent t-test were conducted. The PCA score plot showed a separation between the metabolites profiles of the two groups (R^2X = 0.625, Q^2 = 0.694). The volcano plot also clearly showed significantly different metabolic compounds. Specifically, the prevalence of free fatty acids were increased in EHEC O157:H7 group. Moreover, the intensity of metabolites in the TCA cycle increased in *C. elegans* infected with EHEC O157:H7. Taken together, our metabolomic analysis demonstrate that aerobic metabolism may play important roles in the pathogenicity of EHEC O157:H7, inducing global metabolomic change in *C. elegans*. Our study highlights the applicability of metabolomics on elucidation of pathogenic mechanism.

Keywords : EHEC O157:H7, *Caenorhabditis elegans*, metabolomics

I-6

Massive Screening System for Enumerating Interactions between *Fusarium fujikuroi* and *Oryza sativa* L of Korean Core Set

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Bakanae disease, caused by *Fusarium fujikuroi* Nirenberg, has been one of the most notorious diseases throughout rice-grown regions. To search for resistant germplasms, disease responses of Korean rice core set, composed of 294 rice accessions, were enumerated. In addition, IMI 58289 and B14, representative strains of two pathotypes of *F. fujikuroi*, were employed. Their genomes were also analyzed and uploaded publically. IMI 58289 is the typical type strain producing gibberellic acids during its penetration and infection and induces abnormal seedling elongation of aboveground part. B14 is belonged to an alternative pathotype and produces harmful, phytotoxic secondary metabolites including fumonisin and fusaric acid during its compatible interaction with rice, therefore, B14 inoculation culminated in rice seedling's stunting, withering, and death. *F. fujikuoi* usually infects underground part of rice, therefore, hydroponic seedling culture system was established for the direct *F. fujikuroi* infection into rice roots. Nine sterilized seeds were germinated and grown on the 3 MM filter paper rack in the 6-well plates and in each well, 5 ml Yoshida's solution were added. The inoculum was sprayed on the roots when the seedlings grew 5 cm and the seedling height or number of withered/dead seedlings was measured and counted at 5 days post inoculation. This system is affordable 120 accessions in each trial with two pathotypes.

Keywords : Bakanae, pathotypes, rice

I-7

The Control of Bakanae Disease Caused by *Fusarium fujikuroi* Based on the Interaction of Plant-Drived *Burkholderia* Species with *F. fujikuroi*

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The bakanae disease of rice caused by *Fusarium fujikuroi*, which produced gibberellic acids as major virulence factor, is characterized by hyper-elongation of seedlings. The typical symptoms of bakanae disease are abnormal elongation including tall, lanky tilers, pale green flag leaves, dried-up leaves and infertile panicles. The bakanae disease is the most notorious seed-brone diseases and widespread problems affecting production of rice in the rice-growing countries. The most common management for preventing this disease is treatment of the seeds with hot water or fungicide. Hot water immersion method is ineffective on severely infected rice seed, because it is difficult to reach the pericarp or rice seeds. The application of fungicides is also markedly ineffective for destroying the spores of this fungal pathogen. Also, the control of the bakanae disease has been difficult increasingly due to rapidly developing fungicide resistance in the fungal population. Therefore, with an environmental-friendly approach capable of controlling the bakanae disease with living-microorganisms is effective in continuously controlling the bakanae disease. In this study, identification of the interaction between *F. fujikuroi* and microorganisms using pathogenic and non-pathogenic *Burkholderia* species with rice as host was confirmed by *in vitro* assay, and it has been confirmed in *in vivo* assay with rice plants that the bakanae disease was controlled by its interaction.

Keywords : *Burkholderia* sp. KJ006, *Fusarium fujikuroi*, bakanae disease

I-8

Structure of Flavoprotein RclA from *Escherichia coli*, and Its Molecular Mechanism Contributing to HOCl Resistance

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In response to microbial invasion, the animal immune system generates hypochlorous acid (HOCl) that kills microorganisms in the oxidative burst. HOCl toxicity is amplified in the phagosome through import of the copper cation (Cu^{2+}). In *Escherichia coli* and *Salmonella*, the transcriptional regulator RclR senses HOCl stress and induces expression of the RclA, -B, and -C proteins, involved in bacterial defenses against oxidative stress. However, the structures and biochemical roles of the Rcl proteins remain to be elucidated. Previous study examined the role of the flavoprotein disulfide reductase (FDR) RclA in survival of *Salmonella* in macrophage phagosomes, finding that RclA promotes *Salmonella* survival in macrophage vacuoles containing sublethal HOCl levels. To clarify the molecular mechanism, we determined the crystal structure of RclA from *E.coli* at 2.9 Å resolution. This analysis revealed that the structure of homodimeric RclA is similar to those of typical FDRs, exhibiting two conserved cysteine residues near the flavin ring of the cofactor flavin adenine dinucleotide (FAD). Of note, we observed that Cu^{2+} accelerated RclA-mediated oxidation of NADH, leading to a lowering of oxygen levels *in vitro*. Compared with the RclA wildtype enzyme, substitution of the conserved cysteine residues lowered the specificity to Cu^{2+} or substantially increased the production of superoxide anion in the absence of Cu^{2+}. We conclude that RclA-mediated lowering of oxygen levels could contribute to the inhibition of oxidative bursts in phagosomes. Our study sheds light on the molecular basis for how bacteria can survive HOCl stress in macrophages.

Keywords : Hypochlorous acid, flavoprotein disulfide reductase, crystal structure

I-9

Three Transcriptional Regulators Control *cabABC* Expression Essential for Biofilm Development of *Vibrio vulnificus*

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Biofilms provide bacteria with protection from environmental stresses and host immune defenses. The pathogenic marine bacterium *Vibrio vulnificus* forms biofilms and colonizes environmental niches such as oysters. The *cabABC* operon encodes an extracellular matrix protein CabA and a corresponding type I secretion system, which are essential for biofilm development of *V. vulnificus*. In this study, molecular biological analyses revealed the roles of three transcriptional regulators BrpR, BrpT, and BrpS in the regulatory pathway for the *cabABC* operon. BrpR induces *brpT* and BrpT in turn activates the *cabABC* operon in a sequential cascade, contributing to development of robust biofilm structures. BrpT also activates *brpS*, but BrpS represses *brpT*, constituting a negative feedback loop that stabilizes *brpT* expression. BrpT and BrpS directly bind to specific sequences upstream of *cabA*, and they constitute a feedforward loop in which BrpT induces *brpS* and together with BrpS activates *cabABC*, leading to precise regulation of *cabABC* expression. This elaborate network of three transcriptional regulators BrpR, BrpT, and BrpS thus tightly controls *cabABC* expression, and contributes to successful development of robust biofilms in *V. vulnificus*.

Keywords : *Vibrio vulnificus*, biofilm, gene regulation

I-10

Expression of a MARTX Toxin RtxA Is Controlled by a CRP-Coordinated Regulatory Network in *Vibrio vulnificus*

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A multifunctional-autoprocessing repeats-in-toxin (MARTX) toxin is an essential virulence factor of a fulminating human pathogen *Vibrio vulnificus*. It has been reported that H-NS and HlyU repress and derepress the expression of the MARTX toxin gene *rtxA*, respectively. However, very little is known about other regulatory proteins and environmental signals involved in the *rtxA* regulation. In this study, a leucine-responsive regulatory protein (Lrp) was found to activate *rtxA* by directly binding to the *rtxA* promoter, P_{rtxA}. Phased hypersensitivity resulting from the DNase I cleavage of the P_{rtxA} regulatory region suggests that Lrp probably induces DNA bending in P_{rtxA}. Lrp activates *rtxA* independently of H-NS and HlyU, and leucine inhibits Lrp binding to P_{rtxA} and reduces Lrp-mediated activation. Furthermore, a cyclic AMP receptor protein (CRP) represses *rtxA*, and exogenous glucose relieves CRP-mediated repression. Biochemical and mutational analyses demonstrated that CRP directly binds to the upstream region of P_{rtxA}, presumably altering DNA conformation in P_{rtxA} and thus represses *rtxA*. CRP also represses the expression of *lrp* and *hlyU* by binding directly to their upstream regions. Taken together, a regulatory network consisting of CRP, Lrp, H-NS, and HlyU precisely controls the expression of *rtxA*, in response to changes in host environmental signals such as leucine and glucose, thereby contributing to the fitness and pathogenesis of *V. vulnificus* during the course of infection.

Keywords : *Vibrio vulnificus*, MARTX Toxin, regulatory network

I-11

Cyclo (L-Phe-L-Pro) Passes through Biological Membranes by a Simple Diffusion

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Cyclo (L-Phe-L-Pro)(cFP) is well known to be produced by *Vibrio* species and plays a role as a signaling molecule and a virulence factor as well. Nevertheless, the way how this compound passes across biological membrane remains obscure. Using radiolabeled cFP, we examined the kinetics of transport of this compound across cell membranes using *V. vulnificus*, *E. coli*, and sheep red blood cells. We observed that the radiolabeled cFP was taken up by these cells in a concentration-dependent manner and was not affected by addition of the proton ionophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP). When intracellular accumulation of cFP in these tested cells were measured, the concentration did not show significant differences between 1 min and 10 min time points after cFP was added to the culture. In contrast, the intracellular concentration of fumarate, which is well known to be taken up by cells via active transport, was significantly higher at 10 min than at 1 min time point after addition. Taken together, this study shows that cFP is a diffusible molecule that does not require energy for transportation across biological membranes, and that cFP does not need a membrane machinery to cross membrane to act as a virulence factor or a signal.

Keywords : cFP, simple diffusion, biological membrane

I-12

Screening for the Inhibitor of ToxR, the Receptor of the Cyclic-Phe-Pro Signal, of *Vibrio* Species

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We screened total 6,566 chemicals of the Chembank collection cordially provided by the Korean Chemical Bank to screen candidates that repress the expression of the gene *leuO*, which encode a master regulatory protein for the virulence pathway associated with the signal molecule cyclic-Phe-Pro (cFP), in the human pathogen *Vibrio vulnificus*. For this, we constructed a screening system in which *luxAB*-reporter genes were transcriptionally fused to the promoter region of *leuO*. The construction was introduced into a *leuO*-deletion isotype of *V. vulnificus* strain MO6-24/O. The resulting strain was added with each of the collection chemical dissolved in DMSO, and the Lux activity was quantitatively measured employing DMSO and purified cFP as negative and positive controls, respectively, in 96-well plates. In the 1st round of screening, we selected total 200 chemicals, which showed less than 30% of the Lux activity value comparing to the positive control. In the 2nd round of screening, we narrowed down to 25 chemicals which showed the best scores, and in the 3rd round, we further narrowed down four hits with the best scores. Among these, we selected the candidate 'SGR217-3' and 'SGR217-4'. These compounds inhibit ToxR, an important transmembrane protein associated with ToxR-LeuO-Vhuα-Rpos-KatG pathway in a *V. vulnificus* in concentration-dependent manner. SGR217-3 and 4 also inhibit the expression of *ctxAB* of *V. cholerae*, which also has been known to be a target of the LeuO-signaling pathway. We also observed that SGR217-3 and 4 exerted no significant effect either on growth of *V. vulnificus* cells or viability of human epithelial cell line HaCaT as measured by the MTT assay. We currently are investigating on the remaining hits.

Keywords : *Vibrio vulnificus*, chemical compound, leuO

I-13

Iron-Fur Complex Represses Transcription of Components in the Cyclic-(Phe-Pro) Mediated Regulatory Circuit in *Vibrio vulnificus*

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Vibrio vulnificus, a causative agent for severe lethal septicemia on human host, employs a diketopiperazine compound cyclic(Phe-Pro) (cFP) as a signal molecule. cFP triggers a signal transduction cascade composed of five regulators ToxR, LeuO, vHUa and b, and RpoS in consequence, and each of these components regulates own target genes. Expression of the catalase encoded by *katG* is a target gene regulated by RpoS. We observed that expression of catalase activity is repressed by iron in a Fur (Ferric uptake regulator)-dependent manner. We investigated which step of the signal pathway is regulated by iron, and found that expressions of all of those five functions in the signal pathway as well as *katG* are repressed by iron and its regulation is mediated by Fur. Gel-shift mobility assays showed that Fur-iron binds to the upstream regions of genes encoding these functions. These results together with our previous results showing that Fur-iron represses expression of components of the AI-2 quorum sensing pathway highlights the importance of suppressive roles of iron in pathogenicity of *V. vulnificus*.

Keywords : *Vibrio vulnificus*, fur, leuO

I-14

Elucidation of Molecular Function of Novel Phage Protein Responsible for Optimization of Host Cell Lysis

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Bacteriophages (or phages) are replicated by utilizing the bacterial resources to produce viral proteins and genetic materials and the host cells are destroyed by phage proteins at the final stage of phage replication. In the process, phages use multiple proteins to optimize host cell lysis for maximum phage replication. However, elucidation of the whole phage lysis process is not easy since most phages contain genome replete with numerous uncharacterized genes. Here, we studied the phage-encoded protein involved in the host bacterial cell lysis. Using a random DNA library of phage BSPM4 genome, we found that the overexpressed ORF52 can cause bacterial growth inhibition. Bioinformatic analysis of the ORF52 revealed that it contains 64 amino acids and harbors one transmembrane helix, indicating that the ORF52 can be localized in the bacterial membrane. We constructed the mutant phage lacking the *orf52* (BSPM4 *⊿orf52*) and explore the role of the ORF52 in the host cell lysis process. Surprisingly, BSPM4 *⊿orf52* can replicate much faster than the wild-type phage but the burst size was almost the same as the wild-type phage, suggesting an important role of the ORF52 in host cell lysis by phage. Further investigation of the ORF52 effects on the activity of phage lysis cassettes including holin, endolysin, and spanin revealed that the ORF52 can inhibit the function of holin through direct protein-protein interaction. These results suggest that the ORF52 could modulate the holin function to adjust the phage lysis interval for maximum replication of phage.

Keywords : Bacteriophage, lysis inhibition, anti-holin

I-15

Identification of Insertion-Tolerance Regions in the Small RNA Phage, PP7

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Bacteriophages (phages) have been regarded as valuable platforms for virus-based biopharmaceuticals, especially for display and/or delivery of nucleic acids, proteins, and/or chemical drugs. Most studies, however, have been done for DNA phages rather than RNA phages without the potential drawbacks by phage-mediated lateral transfer of the bacterial genes. This is due to the difficulty of genetic manipulation for RNA phages. We previously developed a reverse genetic system for PP7, a small RNA phage that infects *Pseudomonas aeruginosa* and proved its utility for point mutation analyses of the phage maturation protein (MP) during the phage life cycle. Here, we combined the *in vitro* mutagenesis technique using MuA transposase to identify the insertion-tolerance regions (ITRs), which can accommodate the 15-bp insertion. Eight cDNA clones producing functional phage particles were initially identified from ~1,000 random transposition clones and their insertion sites were identified as the ITRs: three ITRs were mapped within the codons for Ser19 (UC^G) and Ala299 (G^GA) of the MP gene and the between the codons for Gly291 and Lys292 (GGU^AAG) of the RNA-dependent RNA polymerase gene. This result reveals the first ITRs of the RNA phage that can be utilized for tagging RNA and protein/peptide in a way to engineer the RNA phage for pharmaceutical purposes.

Keywords : *Pseudomonas aeruginosa*, RNA phage, phage engineering

I-16

Effect of Subinhibitory Concentrations of Erythromycin on Biofilm Formation and Virulence by *Edwardsiella piscicida*

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Edwardsiella piscicida, the causative agent of edwardsiellosis, is a significant zoonotic pathogen that commonly fatal to freshwater and marine fish. This infectious agent cause tremendous aquacultural economic losses in worldwide. Control of this fish pathogen infection in aquaculture relies chiefly on the use of antibiotic agents. However, the indiscriminate use of antibiotic agents promotes the increase of antibiotic resistance in the aquatic environment. Previous studies have reported that biofilm formation is able to regulate by subinhibitory concentrations (sub-MIC) of antibiotics in a variety of pathogenic bacterium. To understand the environmental signals in biofilm formation of *E. piscicida*, we hypothesized that biofilm of *E. piscicida* can be regulated by sub-MIC of erythromycin which is known for an antibiotic widely used in aquaculture. In sub-MIC of erythromycin, biofilm formation was induced due to increased type 1 fimbrial expression, which lead to increasing of colonization into the host, resulting hyper-virulence. Altogether, indiscriminate use of antibiotics in the aquaculture industry is able to introducing not only multidrug-resistance bacteria but also hyper-virulent strain that can be increase the mortality of fish. In addition, identification of the role of erythromycin as a virulence factor regulatory signals can be lead to us for better understand of *E. piscicida* pathogenesis and prevent of fish borne disease in aquaculture farm.

Keywords : Biofilm, type 1 fimbrial, erythromycin