

D005

Development of SARS Coronavirus (SARS CoV) Quantitive Detection Method with Syber Green-based Real Time PCRSang-Hoon Han^{*}, Do-Bu Lee, Yong-Ho Cho, Jung-Soon Ha, and Byoung-Su Yoon

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The ability to quickly recognize novel human coronavirus (CoV) associated with severe acute respiratory syndrome (SARS) is crucial to prevent spreading of this disease. Since the initial outbreak of SARS, many kinds of detection methods were devised and tried to demonstrate their reliability to detect the causative virus. Among those detection methods, Real-Time PCR assay showed excellency in both rapidity and sensitivity. However, most of them are needed high cost due to detection device and fluorescent dyes. In this study, we tried to invent highly SARS CoV-specific Real-Time PCR detection method with artificially synthesized partial spike protein genes of the virus. This method needs low detection cost comparatively than other Real-Time PCR assay using internal hybridization probe in that detection dye is Syber Green. Determined detection limit of the method was less than 10 copies and amplified SARS CoV-specific PCR product under the condition of presence of contaminants. In conclusion, this detection method might be used in clinical tests for confirm SARS CoV infection.

D006

Hyphae-specific Repression of Catalase is Rescued by Disruption of Thiol-specific of Antioxidant (TSA1) in *Candida albicans*.Saem Jung^{*}, Yeo Jung Kim, Jung Min Ahn, and Wonja Choi

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Previously we identified several proteins that are increased in pathogenic hyphae by comparing protein profiles of yeast and hyphae of *Candida albicans*. One of them, thiol specific antioxidant 1 (TSA1) that is a H₂O₂ scavenger, attracted our attention because it may play some roles in surviving unfavorable oxidative environment created by host cells. When the fungicidal activity of macrophage infected with *C. albicans* cells was examined, most of *tsa1* null mutant cells evaded the phagocytosis by macrophages as early as 3 h post infection, similar to wild-type strain cells. This result suggests either that TSA1 is not involved in the survival from macrophage or that fungicidal activity of macrophage is not derived from the action of intracellular H₂O₂, or both. To reveal the possible relationship of TSA1 with catalase, a major H₂O₂ scavenger, we also examined its expression in yeast and hyphae cells. To our surprise, catalase was expressed in yeast but not in hyphae cells of the wild type, whereas expressed in both cells of *tsa1* null mutant. How hyphae-specific repression of catalase is rescued by disruption of TSA1 and its biological significance are under investigation.

D007

Rapid Identification of Fungal Disease in Honey-bee; *Ascospheara apis*, *Aspergillus flavus*, by Real-Time PCRHye-Min Lee^{*}, Yong-Ho Jo, Jung-Soon Ha, Sang-Hoon Han, and Byoung-Su Yoon

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Chalkbrood and Stonebrood are well known fungal diseases of honeybee, those are caused by the fungus *Ascospheara apis* and by several species of *Aspergillus*, respectively. Because there is no available control method for these kind of disease, and because co-infection with other pathogens should be escaped, the rapid detection system will be important in hive management. In this study, Real-time PCR method for the rapid detection of chalkbrood and stonebrood was developed. Specific primer-pairs were designed based on 18S rRNA gene of *Ascospheara apis* and *Aspergillus flavus*. This PCR system could easily detect the existence of these pathogens from larvae samples. In addition, new universal primers were designed for amplification of 18S rRNA gene from various fungi. It is also available for nested PCR, used by *Ascospheara apis* and *Aspergillus flavus* specific primer-sets.

(Key word : *Ascospheara apis*, *Aspergillus flavus*, Chalkbrood, Real-Time PCR, Stonebrood, 18S rRNA gene,)

D008

Identification and Functional Analysis of *Vibrio vulnificus* SmcR, a Novel Global RegulatorMyung Won Kim^{1*}, Uryung Park¹, Hyun Mok Ju², Na Young Park¹, Hye Sook Jeong¹, Jee Eun Rhee¹, Jeong Hyun Lee¹, and Sang Ho Choi¹¹Department Food Science and Technology, School of Agricultural Biotechnology, and Center for Agricultural Biomaterials, Seoul National University, ²Department of Molecular Biotechnology, Chonnam National University

Microbial pathogenicity is controlled by a common regulatory system. Quorum sensing has been implicated as an important global regulator controlling numerous virulence factors in bacterial pathogens. In the present study, an open reading frame, *smcR*, a homologue of *V. harveyi* LuxR, was identified from *V. vulnificus*. The deduced amino acid sequence of SmcR from *V. vulnificus* was 72 to 92% similar to those of LuxR homologues from *Vibrio* spp. Functions of the SmcR were assessed by an isogenic mutant, and by evaluating its phenotype changes *in vitro* and in mice. The disruption of *smcR* resulted in a decrease and an alteration in growth rate, biofilm, morphology, and motility. The *smcR* mutant exhibited reduced survival under acidic pH and hyperosmotic stress, and decreased cytotoxicity and adherence toward EL4 T cells *in vitro*. Furthermore, the LD₅₀ of the *smcR* mutant was approx 10² times higher than wild type. Therefore, it appears that SmcR is a novel global regulator contributing to pathogenesis as well as survival of *V. vulnificus*. [Supported by grants from the 21C Frontier Microbial Genomics and Application Center Program]