D005

Development of SARS Coronavirus (SARS CoV) Quantitive Detection Method with Syber Green-based Real Time PCR

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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 thosedetection 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 VoV-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 confir SARS CoV infection.

D006

Hyphae-specific Repression of Catalase is Rescued by Disruption of Thiol-specific of Anti-oxidant (*TSA*) in *Candida albicans*.

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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 H_2O_2 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 H_2O_2, or both. To reveal the possible relationship of *TSA1* with catalase, a major H_2O_2 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; *Ascosphera apis, Aspergillus flavus*, by Real-Time PCR

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Chalkbrood and Stonebrood are well known fungal diseases of honeybee, those are caused by the fungus *Ascosphaera 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 *Ascosphaera apis* and *Aspergillus flavus* This PCR system could easy detect the existence of these pathogens from larvae samples In addition, new universal primers was designed for amplification of 18S rRNA gene from various fungi It is also available for nasted PCR, used by *Ascosphaera apis* and *Aspergillus flavus* specific primer-sets

(Key word . *Ascosphaera apis, Aspergillus flavus,* Chalk-brood, Real-Time PCR, Stonebrood, 18S rRNA gene,)

D008

Identification and Functional Analysis of Vibrio vulnificus SmcR, a Novel Global Regulator

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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 homlogues 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 LD_{50} of the *smcR* mutant was approx 10^2 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*

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