Screening of Monoclonal Antibodies Against a Viral Protein Using Protein Chip Technology

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Oyster mushroom spherical virus (OMSV) is a causative agent of Die-back disease in oyster mushroom, Pleurotus ostreatus. Biochemical characterization of OMSV has revealed that it consisted of a single-stranded RNA genome that encodes at least 7 open reading frames including a viral coat protein and an RNA-dependent RNA polymerase (RDRP). RDRP is the crucial and specific catalytic enzyme for viral replication. To further study the viral replication process in its host mushroom cells, we have produced monoclonal antibodies (MAbs) against a fragmented RDRP protein. For the rapid screening of MAb, a protein chip technology based on Alexa-488(A488) dye labeling method was introduced. Eighty seven monoclonal antibodies (MAbs) against the fragmented RDRP protein (F7) were generated from mouse hybridoma cells. The F7 protein was chemically coupled onto an amine-modified slide glass. The MAbs were spotted onto the F7-coupled slide glass. The amounts of bound MAbs were measured by binding of A488-modified secondary antibody using a fluorescent image scanner. Five out of 87 MAbs have chosen by the signal intensity. The specificity of the selected MAbs in detecting OMSV RDRP was further justified by an immunoblot analysis and a surface plasmon resonance biosensor assay.