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Application of Monoclonal Antibody Technique to Serological Studies on Virus and Virus-like Diseases of Plants

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植物의 바이러스 및 類似 바이러스病의 血清學的 研究의 모노크로날 抗体方法의 應用

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Monoclonal antibody technique has been applied to indentify the strains of the important viruses and causal agents causing virus-like diseases of crops in Taiwans in order to monitor the epidemiological dynamic of the pathogens in the field, and index the propagative materials during plant quarantine, since these investigations have been hardly accomplished by means of the conventional serological methods.

Various strains of papaya ringspot virus (PRSV) have been seriously invading papaya plants in Taiwan, and field trials of controlling the disease have been made by cross protection. An attempt was made to prepare monclonal antibodies specific to the different virus strains for monitoring the mild protective strain and severe challenging strains in the field. Hybridoma clones secreting specific monoclonal antibodies against the three strains of PRSV were obtained by fusing murine myeloma cells (NSI) with splenic cells from BALB/C mice immunized with purified virus preparation of mild mottle strain (M) or necrotic severe mottle strain (SMN) replicated in Zucchini squash. The antigenic specificity of the monoclonal antibodies (McAbs) was analyzed with the four differential virus strains by indirect enzyme-linked immunosorbent assay (ELISA) with alkalin phosphatase-linked antimouse secondary antibodies or direct ELISA with enzyme-linked McAbs against the virus strains. Nineteen stable cell lines of 24 hybridoma lines secreting specific monoclonal antibodies against papaya ringspot virus, were screened and cloned. The monoclonal antibody lines were differentiated into 4 groups. Group 2 McAbs strongly reacted with the three strains of papaya, but not with WMV-1 strain of cucumber, might derive from the common antigenic determinant on virus particles of the all strains. Group 3 McAb was monospecific to SM-strain, and Group 1 and Group 4 showed dispecific to SM- and M-strains, however the latter reacted with SM-strain more slightly than the former. Isotyping of these monoclonal antibodies was made by agar double immunodiffusion with isotyping kit (Zymed Co.), and they were of IgG2a, IgG2b or IgGl isotypes respectively. Antibody titers for both hybridoma culture supernatants and ascitic fluids of McAb-12 determined with indirect ELISA, were 1,000 and 100,000 respectively, and those of McAb-14 ranged 1,000 and 1,000,000, respectively. The dilution end-points of virus antigen for positive

eaction with McAb-12 and McAb-14 determined y indirect ELISA, were 1,000X and 8,000X, espectively. Purified monoclonal antibody pre- aratin of high purity was obtained by filtration nrough Protein A-Sepharose CL-6B column with ml of ascitic fluid. Enzyme-linked immunoglobulin conjugate) with titer as high as 2,500X, was pre- ared by conjugating alkaline phosphatase with urified antibody of McAb-14, and the conjugate vas applied to direct ELISA. Specific fluorescence ppeared in the sections of papaya and zucchini eaves infected with the different strains of PRSV n indirect immunofluorescence staining with scitic fluid (200X) or purified antibody(40X). The pplication of monoclonal antibody to serological pecific immunoelectronmicroscopy revealed trapp- ng effectiveness of supporting film on grid and top- lecoration of virus particles.

Hybridoma technique has been also applied to roducing monoclonal antibodies against the virus ausing bunchytop, a destructive and common lisease of banana plants in order to facilitate serolo- ical study on the virus entity, and establish a rapid nd accurate method for indexing propagative naterials and banana seedlings. Some advances nd results have been obtained. D strain of sugarcane nosaic virus (SMV) caused serious damage to edible Bdila sugarcane, and some industrial sugar- cane varieties on this island. McAbs reacted with the strains of SMV were also prepared through the hybridoma technique. The monospecificity of the McAbs will be identified further in order to obtain the McAbs specific to SMV-D strain for indexing seed canes free from the destructive D strain.

The mycoplasma-like organisms (MLOs) causing paulownia witches' broom and cucurbitaceous witches' broom were successfully transmitted to periwinkle plants via dodder vines. The MLOs were found to propagate considerably well in periwinkle and dodder, and MLO preparations were obtained through isolation and purification from the both infected plants. Trials of producing McAbs against the MLOs have been made by means of hybridoma technique with BALB/C mice immuniz- ed with the MLO preparations from dodder, and screening hybridoma cell lines with MLO prepara- tions from periwinkles or the diseased host plants. An attempt has been made to prepare McAbs against the pathogenic agent, fastidious bacteria causing likubin (greening) disease of citrus which has been devastating citrus trees in Taiwan. Some encouraging results has been obtained. The pro- blems encountered during the present hybridoma works will be discussed.