European Journal of Plant Pathology **101:** 511–518, 1995.

Monoclonal antibody for the detection and identification of a phytoplasma associated with rice yellow dwarf

Fu-Lin Chang[1], Ching-Chung Chen[2] and Chan-Pin Lin[1]*
[1]*Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan 107, R.O.C.;*
[2]*Taichung District Agricultural Improvement Station, Changhua, Taiwan 515, R.O.C.;*
**Author for correspondence*

Accepted 28 February 1995

Key words: immunodiagnostics, tissue-blotting techniques

Abstract

One stable hybridoma clone, 247B11, secreting specific monoclonal antibody (MA) against the mycoplasmalike organism (MLO), newly be termed phytoplasma, associated with rice yellow dwarf (RYD) was produced by employing an immunization scheme for inducing the immunological tolerance of mice to rice antigens prior to the administration of RYD-phytoplasma immunogens. Neonatal BALB/c mice were first injected with nontarget rice antigens present in the immunogen preparation and were immunized intrasplenically with RYD-phytoplasma-enriched antigens prepared by Percoll density-gradient fraction 6 wk later. The MA was of the IgG1 class. With this MA, RYD-phytoplasma in diseased rice was specifically detected by indirect enzyme-linked immunosorbent assay (ELISA), immunofluorescent staining and tissue-blotting techniques. Antibody titer determined by indirect ELISA for hybridoma-culture supernatant was 5120. The antibody recognized two polypeptides, 16 kDa and 41 kDa, of RYD-phytoplasma determined by western blotting. RYD-phytoplasma was differentiated serologically from the phytoplasmas associated with sweetpotato, peanut, loofah, paulownia, and *Ipomoea obscura* witches' broom, aster yellows (NJ strain), elm yellows, and sugarcane white leaf both in indirect ELISA and immunofluorescent staining.

Introduction

Rice yellow dwarf (RYD) disease, caused by a mycoplasmalike organism (MLO, newly be termed pytoplasma), is widespread in rice-growing Asian countries. The disease agents are transmitted by the green rice leafhoppers *Nephotettix cincticeps* Uhler, *Nephotettix nigropictus* Stal, and *Nephotettix virescens* Distant [Ou, 1985]. RYD-phytoplasma-infected plants exhibit characteristic symptoms such as chlorosis, yellowish-green leaves, stunting of plants, and excessive tillering. The symptoms are conspicuous on the regenerated growth when the plants are ratooned.

RYD, discovered around 1932 in Taiwan [Kurosawa, 1940], caused great losses particularly in the second crop of the years after the 1960's. Although the incidence of RYD disease was generally low during recent years, this disease occasionally caused serious damages to the rice crop in the Nantou, Changhua, and Chiayi areas of Taiwan.

Like other yellows disease agents, the phytoplasma associated with RYD disease has not been cultured *in vitro*. Diagnosis based on symptomatology, electron microscopy of ultra-thin sections, and inset transmission are difficult and time-consuming. For immunodiagnostics, polyclonal antibodies for various phytoplasmas now available have relatively low specific titers and exhibit considerable nonspecific reactions due to the host contaminants present in the phytoplasma immunogens. Polyclonal antibodies for RYD-phytoplasma produced recently using antigen preparations from diseased rice plants encountered the same difficulties [Onuki *et al.*, 1992]. In the past ten years, only a few specific monoclonal antibodies (MAs) against a limited number of phytopathogenic phytoplasmas were developed and applied in the dis-

ease detection after the fist report appeared [Lin and Chen, 1985].

In this paper, we report the production of MA specific to RYD-phytoplasma by employing an immunization scheme that included the induction of immunological tolerance to plant antigens in mice prior to the intrasplenic administration of RYD-phytoplasma-enriched immunogens. The serological relatedness among various phytopathogenic phytoplasmas were determined by ELISA and by immunofluorescent staining with the MA. Tissue blotting and western blotting were also applied to detect RYD-phytoplasma and RYD-phytoplasma-specific polypeptides.

Materials and methods

RYD-phytoplasma-infected plants. Rice (*Oryza sativa* L.) plants naturally infected with RYD-phytoplasma were collected from fields in the Nantou, Changhua, and Chiayi areas of Taiwan. The disease agents were maintained in rice plants, variety Tainan 5, in the greenhouse at the Taichung District Agricultural Improvement Station. The RYD-phytoplasma was inoculated into healthy rice plants for 3 days by green leafhoppers (*N. cincticeps*) which had been infected by a 3-day access feeding on RYD-infected rice plants at 26–28 °C and then a 4-wk feeding on healthy rice for the multiplication of the phytoplasma in the green rice leafhoppers.

Antigen preparation. Fourfold (1 ml of antigen suspension derived from 4 g of fresh leaf midribs) concentrated stock solutions were prepared from leaf midribs of RYD-phytoplasma-infected rice and healthy rice. Leaf midribs were ground in isolation medium [Jiang *et al.*, 1988] and the homogenate was subjected to two cycles of differential centrifugation (2,000 g for 10 min and 15,000 g for 40 min). The pellet was suspended in 0.02 M phosphate-buffered saline (PBS, pH 7.2) as concentrated stock solutions. The stock solution of phytoplasma-enriched antigen was used to screen hybridomas, and to evaluate detection sensitivities and ELISA titers of antibodies. The stock solution of healthy plant extract was used as the immunogen for inducing tolerance of mice to nontarget antigens [Hsu *et al.*, 1990]. Additionally, a method employing Percoll density-gradient fractionation, modified from the protocol described by Jiang and Chen [1987], was applied to prepare another type of phytoplasma immunogen as described previously [Shen and Lin, 1993].

Immunization. The immunization scheme for hybridoma production was adopted from the method reported earlier [Hsu *et al.*, 1990; Shen and Lin, 1993; Spitz *et al.*, 1984]. Neonatal BALB/c mice were injected intraperitoneally with 20 μl of healthy plant antigens within 24 h after birth. The second and the third intraperitoneal injections were made on days 4 and 7, respectively with 20 μl of the same preparations for inducing the immunological tolerance of mice to rice antigens. When the mice were 6-wk-old, they were administered with an intrasplenical immunization [Spitz *et al.*, 1984], and the injection contained 200 μl of the partially purified RYD-phytoplasma antigen prepared by the Percoll density-gradient method. Their spleens were removed for cell fusions 3 days after the final injection.

Monoclonal antibody production. The procedure for splenic cell collection, fusion with murine myeloma cells (P3-NS1/1-Ag4–1), and cultivation of putative hybridoma cells in RPMI complete medium was similar to those described previously [Lin and Chen, 1985; Shen and Lin, 1993]. The culture fluids from wells containing hybridomas were screened for the presence of RYD-phytoplasma-specific antibodies by indirect ELISA with the alkaline phosphotase enzyme system after the procedure described previously [McLaughlin *et al.*, 1989; Shen and Lin, 1993]. The coating antigens used were prepared by the differential centrifugation method as described in antigen preparation, but were diluted 16-fold in 0.05 M carbonate buffer (pH 9.6) [Shen and Lin, 1993] from the fourfold concentrated stock solutions. Hybridomas that secreted antibodies reacting with diseased but not with healthy plant preparations were selected for further cloning by limiting dilution [Halk, 1984]. Monocloned hybridoma cells were subcultured for antibody production and stored in liquid nitrogen for further use.

Antibody isotype determination. Antibody class was determined by indirect ELISA with the mouse monoclonal subisotyping kit (Hyclone Laboratories, Inc., Logan, Utah), according to the vendor's instructions. Monoclonal antibodies used in this study were harvested from hybridoma culture supernatants when cell titers reached 5×10^8 cells ml^{-1} and were used undiluted.

ELISA titer of antibody. Indirect ELISA with an alkaline phosphotase system was used in the ELISA titer tests of MAs. Monoclonal antibodies used in the study also were harvested from hybridoma culture supernatants, as in antibody isotype determinations. A 1:2 dilution series of culture supernatants prepared in 0.02 M PBS (pH 7.2) was tested with a minimum dilution of 1:15. Coating antigens used in the tests were prepared from the leaf midribs of both RYD-phytoplasma-infected and healthy rice by the differential centrifugation method as described in antigen preparation, and the coating antigens applied in the test were diluted 128-fold in carbonate buffer from the fourfold concentrated stock solutions. ELISA titer of antibody was determined as the greatest dilution of antibody that gave a positive ELISA reaction in which the absorbance value at 405 nm was greater than 0.1.

Antibody sensitivity test. Indirect ELISA also was used to determine the detection sensitivity of the RYD-phytoplasma MAs for RYD-phytoplasma in diseased rice plants. Monoclonal antibodies were harvested from hybridoma culture supernatants and were used undiluted. The antigen preparations at various dilutions were obtained from the midrib tissues of both RYD-phytoplasma-infected and healthy rice by the differential centrifugation method as described previously. For the antigen preparations used in this study, a 1:2 dilution series was prepared in carbonate buffer starting with a minimum dilution at a concentration of antigen diluted 4-fold from the fourfold concentrated stock solution. The results of the antibody sensitivity test were presented by the minimal concentration of antigen preparation that reacted with the MA and produced an A_{405nm} value greater than 0.1.

Immunofluorescent staining. Cross and longitudinal sections of leaf midribs were prepared from healthy and RYD-phytoplasma-infected rice by freehand sectioning. Indirect immunofluorescent staining was carried out by following the procedure described previously [Shen and Lin, 1993]. Stained samples were mounted on microslides with glycerol-PBS (9:1, v/v) and examined with an Olympus epifluorescence microscope with an USH-102D 100-W high-pressure mercury lamp and a combination of filters (a BP-495 excitation filter, a BH2-DMIB dichroic mirror, and a G520 barrier filter) to generate an excitation with the main wavelength at 495 nm. Photomicrographs were made with a 35-mm Olympus automatic photomicrographic system (PM-10ADS) on ASA 400 film (Kodak Tri-X Pan, or Ektachrome).

Antibody specificity. Indirect ELISA and immunofluorescent staining were used to test the specificity of RYD-phytoplasma MA and to reveal the serological relatedness among various phytopathogenic phytoplasmas. Nine phytoplasma preparations tested in this study were prepared from diseased plants of rice yellow dwarf, sweetpotato, peanut, loofah, paulownia, and *Ipomoea obscura* witches' broom, aster yellows (New Jersey strain), Elm yellows, and sugarcane white leaf (kindly provided by CT Chen, Taiwan Sugar Research Institute, Tainan, Taiwan). Among these, phytoplasma preparations from diseased plants of rice yellow dwarf and sugarcane white leaf were prepared from original hosts; all the others were prepared from infected periwinkle. Test antigens for ELISA were prepared from healthy and phytoplasma-infected plants in the same manner, as the preparation of partially purified coating antigens from whole leaves; the coating antigens were diluted 16-fold in carbonate buffer from the fourfold concentrated stock solutions. Cross sections of leaf midribs were prepared for the immunofluorescent staining from healthy and phytoplasma-infected plants.

Immunological detection of antigens on tissue blots. The fresh rice tissues were cut crosswise and longitudinally with a new razor blade, and the tissue imprints were obtained by pressing the newly cut surface onto nitrocellulose (NC) membrane with a pore size of 0.45 μm (Schleicher and Schuell, Inc., Keene, NH). The membranes were then treated with the similar indirect immunological method reported by Lin *et al.* [1990] for the detection of RYD-phytoplasma antigens. After blocking with 3% non-fat dry milk, the NC membrane was then incubated with undiluted hybridoma culture supernatant. This was followed by reaction with alkaline phosphatase conjugated goat antimouse *IgM + IgG* (Jackson Immuno Research Laboratories, Inc., West Grove, PA). The blots were finally visualized by incubating the membranes in the dark in a solution containing colorimetric substrate, nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) [Ko and Lin, 1994]. Upon color development, blots were examined and photographed directly or under a stereo microscope.

SDS-PAGE. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted

with a discontinuous homogeneous gel having 4% acrylamide in the stacking gel and 12% acrylamide in the resolving gel. The SDS-PAGE was performed in a cooled dual slab unit (SE600, Hoefer Scientific Instruments, San Francisco, CA) by the method of Laemmli [1970]. Before electrophoresis, 50 μl partially purified rice sample (diluted 4-fold in carbonate buffer from fourfold concentrated stock solutions) was mixed with equal volumes of sample buffer (0.125M Tris-Cl, pH 6.8, 20% glycerol, 4% SDS, 10% 2-mercaptoethanol, and 0.1% bromophenol blue) and boiled for 90 sec. Samples were electrophoresed at 20 mA for stacking gel and 30 mA for resolving gel, respectively for a total of 4h at 14 °C. SDS-PAGE broad range molecular weight standard markers (Bio-Rad Laboratories, Inc., Hercules, CA) were loaded onto adjacent lanes. Gels were stained with silver stain kit (BIO-Rad Laboratories, Inc.) following the vendor's directions.

Western blotting and protein detection. Electrophoretically separated proteins on the stainless slab gel were transferred to the NC membranes (Schleicher and Schuell, Inc.) by a semi-dry transfer unit (TE-77, Hoefer Scientific Instruments), according to the manufacturer's instructions. After proteins being transferred, the membranes were treated with the immunological method described previously for the detection of RYD-phytoplasma antigens in tissue blots. The NC membranes were then rinsed with water to stop the reaction when signals appeared.

Results

From six independent fusions, five hybridoma clones that secreted antibodies specific for RYD-phytoplasma were selected. One of the five hybridomas was stable to sustained subculturing. The stable hybridoma clone, 247B11, was obtained from mice immunized intrasplenically with immunogen prepared from RYD-phytoplasma-infected rice by Percoll density-gradient fractionation. This MA was of the IgG1 class.

Antibody ELISA titers of hybridoma-culture supernatant for 247B11 was 5,120, when measured against RYD-phytoplasma-enriched diseased preparations diluted 128-fold from the fourfold concentrated stock solution. In the antibody sensitivity test, the MA from 247B11 detected the phytoplasma antigen presented in antigen preparations diluted as high as 8,192-fold from the fourfold concentrated stock solution readily.

Representative micrographs showing the results of immunofluorescence tests on the cross and longitudinal sections of midribs from healthy and RYD-phytoplasma-infected rice stained with MA are shown in Figure 1. Only phloem elements of the diseased section packed with RYD-phytoplasma showed FITC-specific apple-green fluorescence (Fig. 1B and D), whereas those of healthy sections did not show any FITC-specific fluorescence (Fig. 1A and C). Other areas beside phloem tissues from both healthy and diseased plants exhibited brownish or greenish yellow autofluorescence that could be easily differentiated from specific apple-green immunofluorescence (Fig. 1). Neither healthy nor diseased sections treated with RPMI complete medium, MAs for sweetpotato witches' broom (SPWB) phytoplasma [Shen and Lin, 1993], or pre-immune mouse serum instead of MA for RYD-phytoplasma, in the antibody treatment step exhibited any FITC-specific fluorescence.

In the antibody specificity test, the MA reacted specifically in ELISA and in immunofluorescent staining with RYD-phytoplasma-infected but not with healthy rice tissues. The antibody did not react either in ELISA or in immunofluorescent staining with preparations from plants infected with phytoplasmas that cause sweetpotato, peanut, loofah, paulownia, and *I. obscura* witches' broom, aster yellows (NJ strain), elm yellows, and sugarcane white leaf.

In tissue-blotting test, the RYD-phytoplasma antigen was readily detected in the phloem tissues. Stem-tissue blots printed by the RYD-phytoplasma infected rice showed the blue-purple color reaction in the areas corresponding to the phloem tissue (Fig. 2B and D). On the other hand, there was no color development in tissue blots printed by uninfected healthy rice plants (Fig. 2A and C).

An SDS-PAGE profile of protein from RYD-diseased and healthy rice is shown in Figure 3. It is very difficult to identify the specific antigenic protein bands associated with the phytoplasma diseases from the results obtained with silver staining of SDS-PAGE electrophoresis gels (Fig 3A). Therefore, the blotting on nitrocellulose membrane and the immunoprobing with specific antibodies is essential for the determination of the antigenic proteins of the RYD-phytoplasma. When protein profiles from SDS-PAGE were transferred to NC membranes and probed with MA from 247B11, two RYD-phytoplasma specific polypeptides bands, 16 and 41 kDa in molecular weight, were seen only in the blots of protein profiles from the diseased rice (Fig. 3B). Apparently, these two polypep-

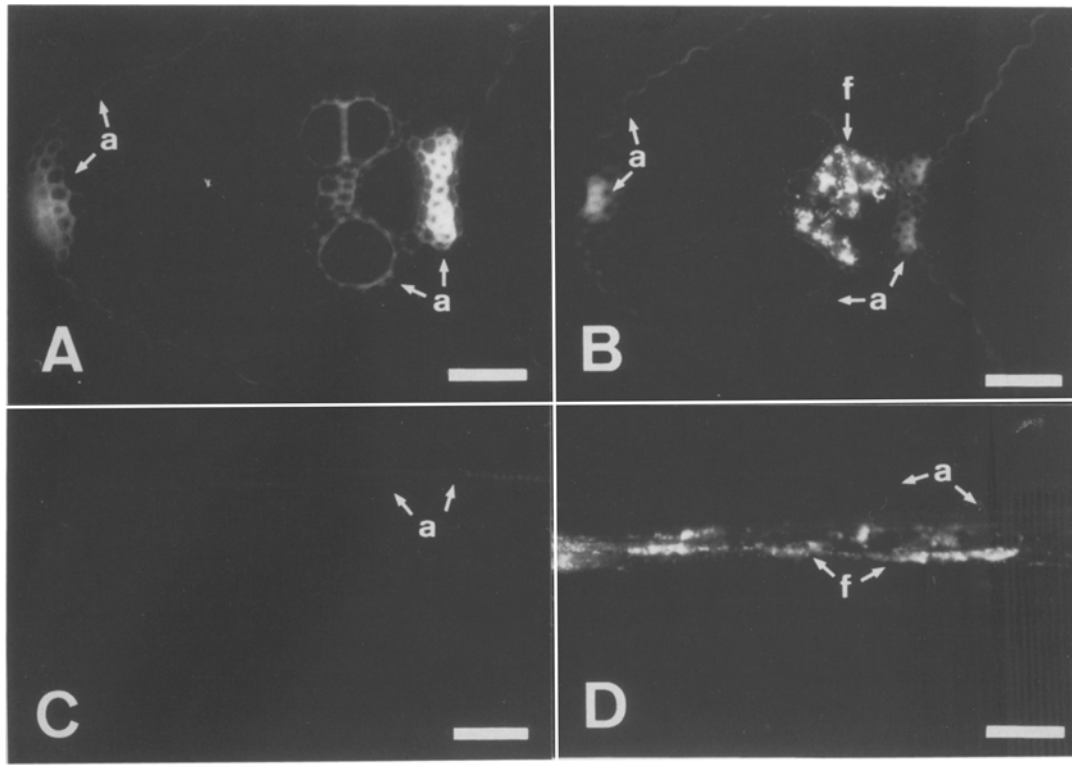

Fig. 1. Immunofluorescent staining on cross and longitudinal sections of leaf midribs from healthy and diseased rice reacted with the monoclonal antibody for rice yellow dwarf phytoplasma from undiluted culture supernatant of hybridoma clone 247B11. A, cross section of healthy rice; B, cross section of diseased rice; C, longitudinal section of healthy rice; D, longitudinal section of diseased rice; A and C, respectively, showing the greenish yellow autofluorescence (a); B and D, respectively, showing the fluorescein isothiocyanate-specific apple-green fluorescence (f) in phloem tissues, and the greenish yellow autofluorescence in other areas of the sections. Bars = 50 μm.

tide bands from RYD-phytoplasma contain the epitopes recognized by the MA for RYD-phytoplasma. No polypeptide band was detected in the blots from healthy plants.

Discussion

For RYD-phytoplasma, DNA probes for different geographical cell lines were developed in our lab [Wu *et al.*, 1993] and in Japan [Nakashima *et al.*, 1993] and applied in the identification of the phytopathogenic organism. However, from our experiences in screening field-collected samples, we realized that not all laboratories, at least for the time being in most developing countries, are well equipped to use nucleic acid probes or related techniques such as polymerase chain reactions. The use of MAs provided a precise method for rapid detection of phytoplasmas *in vitro* and *in situ* with competitive sensitivities [Fos *et al.*, 1992; Hsu *et al.*, 1990; Jiang *et al.*, 1989; Ko and Lin, 1994; Shen and Lin, 1994]. Approaches including indirect ELISA, immunofluorescent staining and tissue-blotting had been proved to give most promising results when MAs were used for the disease detection. Among these approaches, tissue-blotting techniques used in this study and previous report [Ko and Lin, 1994; Shen and Lin, 1994] could be the most efficient and applicable method for the onsite detection of the phytoplasma diseases especially in epidemiological studies. It allows a large number of sample to be screened simultaneously in a very short period of time. It is now adopted in our lab for the inspection of phytoplasma infection, screening of phytoplasma-free propagating tissues and also for the ecological studies of sweetpotato witches' broom (SPWB), peanut witches' broom, and RYD-disease. Recently, we also discovered that tissue-blotting techniques incorporated with

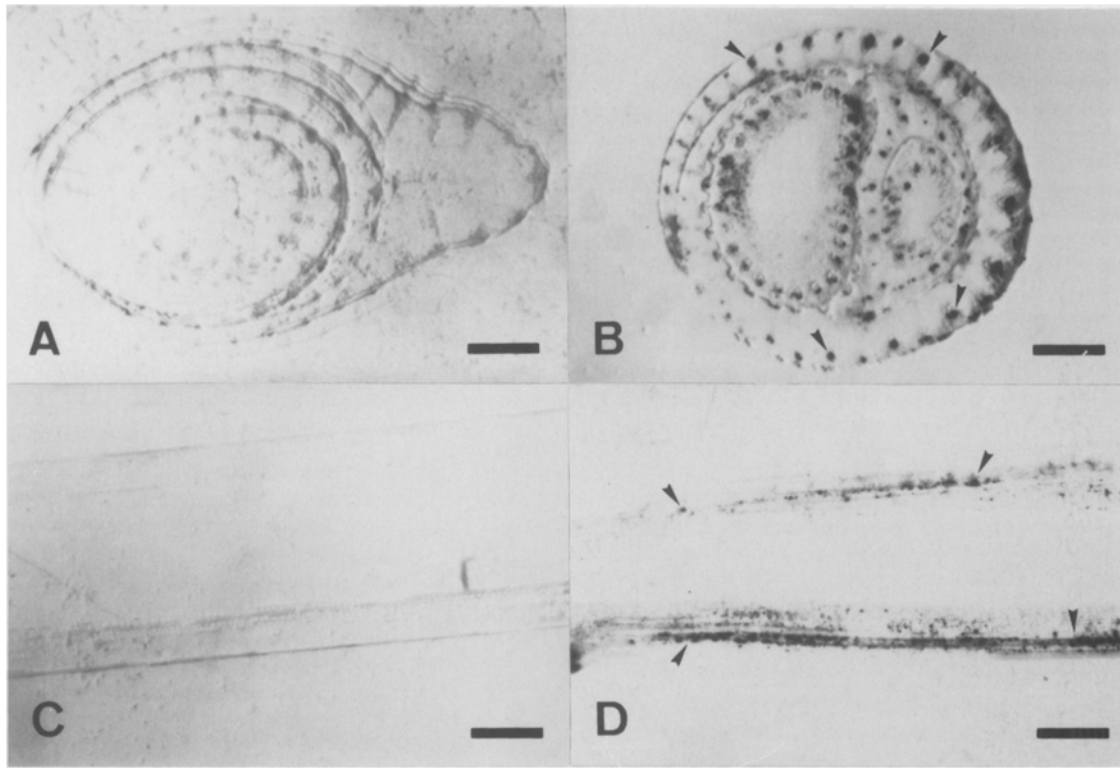

Fig. 2. Immunochemical detection of rice yellow dwarf (RYD) phytoplasma in tissue blots of infected stem of rice on nitrocellulose membranes. The blots were reacted with RYD-phytoplasma monoclonal antibody from undiluted culture supernatant of hybridoma clone 247B11 and detected with alkaline phosphatase-labeled goat anti-mouse immunoglobulin antibodies. A, cross section of healthy rice; B, cross section of diseased rice; C, longitudinal section of healthy rice; D, longitudinal section of diseased rice; B and D, respectively, showing the specific blue-purple color reaction product in areas on the blots (arrows) corresponding to the position of the phloem in rice stems. Bars = 1 mm.

MAs could be the only effective serological approach for the detection of *Xylella fastidiosa*, the causal agent of marginal scorch of pear in Taiwan, that presented sparsely in the xylem tissues of woody pear trees (C. P. Lin, unpubl.).

In this study, the tolerance-inducing procedure along with the intrasplenic immunization strategy was applied for the production of MAs for RYD-phytoplasma. The facility of intrasplenic immunization seems to be due to the deposition of the immunogen directly into the spleen, thus avoiding the losses associated with systemic injections, and to the immobilizing of the immunogen within the spleen, thus prolonging its exposition to the antigen-presenting cells [Spitz *et al.*, 1984]. The intrasplenic immunization strategy could be useful for developing MAs against minute amounts of target antigens present in a mixture of other immunogens. Since RYD-phytoplasma can not be maintained in any alternative host, rice is thus the only plant host to keep the pure line of RYD-phytoplasma by tedious inoculation procedure through insect-feeding. To prepare the RYD-phytoplasma antigens from rice plants to desired purity in this study is more difficult than to prepare other phytoplasma antigens from alternate propagating host plants such as periwinkle (*Catharanthus roseus* (L.) G. Don) in the previous study [Shen and Lin, 1993]. The intrasplenic immunization scheme was consequently proved to be helpful in this study.

The MA specific to RYD-phytoplasma was currently applied for the investigation of the distribution of RYD-phytoplasma in various parts of insect vectors, and infected rice with immunofluorescent staining and tissue-blotting techniques. Meanwhile, we have also excised the strips from the phytoplasma-specific polypeptide bands in western blotting membranes to forward the production of phytoplasma-specific polyclonal antibodies. Such antibodies can

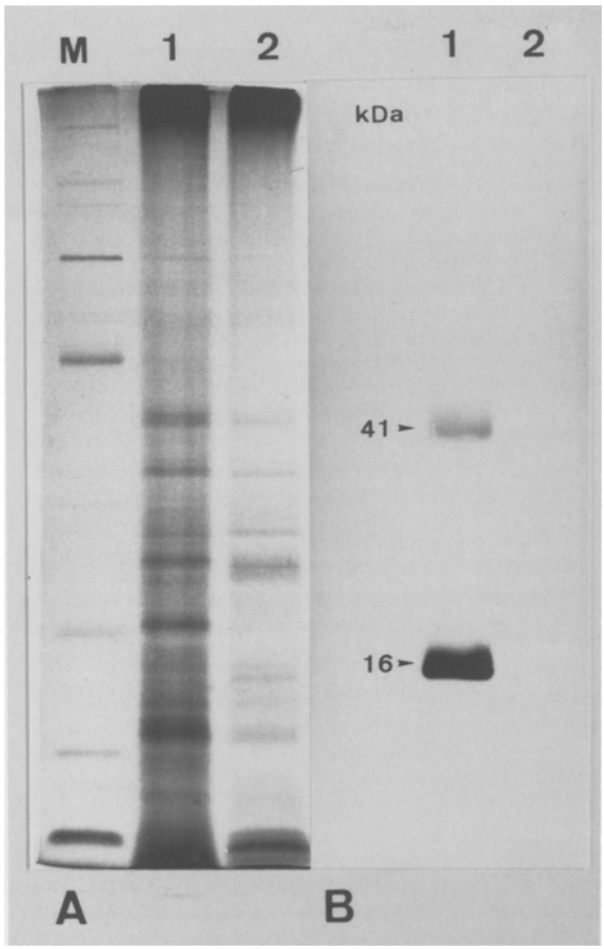

Fig. 3. A, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of proteins (in a 12% polyacrylamide gel) from rice yellow dwarf (RYD)-diseased and healthy rice plants: lane M, SDS-PAGE broad range molecular weight standards 200, 116.3, 97.4, 66.2, 45, 31, 21.5, 14.4 and 6.5 kDa (Bio-Rad); lane 1, preparations from RYD-phytoplasma infected rice plants; lane 2, preparations from healthy rice plants. B, western blot of the SDS-PAGE protein profiles of A probing with monoclonal antibody from clone 247B11. Two polypeptides, 41 kDa and 16 kDa, from diseased rice preparations were recognized by the RYD-phytoplasma monoclonal antibody.

further help in the study of specific RYD-phytoplasma proteins and maybe their receptors in plant and insect hosts. Recently, we have also successfully cloned and sequenced the antigenic gene responsible for the 18.4 kDa membranous protein of SPWB-phytoplasma by using SPWB-phytoplasma-specific MAs in the screening of SPWB-phytoplasma genomic library (CP Lin, unpubli.). The 18.4 kDa protein is now expressed *in vitro* and used as the immunogen for the production of SPWB-phytoplasma specific polyclonal antibodies. The relationships among the major membranous antigens of different phytoplasmas [Clark *et al.*, 1989; Jiang *et al.*, 1988; Onuki *et al.*, 1992] probed with various species of antibodies in western blotting could be then studied.

Acknowledgements

We thank W. H. Ko, Taichung District Agricultural Improvement Station, Changhua, Taiwan, for providing healthy and phytoplasma-infected rice plants, and Y. L. Yu for critical review of the manuscript. This research was supported in part by grant 82 AT-1.1-FAD-56(7) from the Council of Agriculture, R. O. C.

References

Clark MF, Morton A and Buss SL (1989) Preparation of mycoplasma immunogens from plants and a comparison of polyclonal and monoclonal antibodies made against primula yellows MLO-associated antigens. Ann. Appl. Biol. 114: 111–124

Fos A, Danet JL, Zreik L, Garnier M and Bove JM (1992) Use of a monoclonal antibody to detect the stolbur mycoplasmalike organism in plants and insects and to identify a vector in France. Plant Dis. 76: 1092–1096

Halk EL, Hsu HT, Aebig J and Franke J (1984) Production of monoclonal antibodies against three ilarviruses and alfalfa mosaic virus and their use in serotyping. Phytopathology 74: 367–372

Hsu HT, Lee I-M, Davis RE and Wang YC (1990) Immunization for generation of hybridoma antibodies specifically reacting with plants infected with a mycoplasmalike organism (MLO) and their use in detection of MLO antigens. Phytopathology 80: 946–950

Jiang YP and Chen TA (1987) Purification of mycoplasma-like organisms from lettuce with aster yellows disease. Phytopathology 77: 949–953

Jiang YP, Lei JD and Chen TA (1988) Purification of aster yellows agent from diseased lettuce using affinity chromatography. Phytopathology 78: 828–831

Jiang YP, Chen TA, Chiykowski LN and Sinha RC (1989) Production of monoclonal antibodies to peach eastern X-disease agent and their use in disease detection. Can. J. Plant Pathol. 11: 325–331

Ko HC and Lin CP (1994) Development and application of cloned DNA probes for a mycoplasmalike organism associated with sweetpotato witches'-broom. Phytopathology 84: 468–473

Kurosawa E (1940) On rice yellow dwarf disease of rice occurring in Taiwan. J. Plant Prot. 27: 161–166

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Lin CP and Chen TA (1985) Monoclonal antibodies against the aster yellows agent. Science 227: 1233–1235

Lin NS, Hsu YH and Hsu HT (1990) Immunological detection of plant viruses and a mycoplasmalike organism by direct tissue blotting on nitrocellulose membranes. Phytopathology 80: 824–828

McLaughlin RJ, Chen TA and Wells JM (1989) Monoclonal antibodies against *Erwinia amylovora*: Characterization and evalua-

tion of a mixture for detection by enzyme-linked immunosorbent assay. Phytopathology 76: 610–613

Nakashima K, Kato S, Iwanami S and Murata N (1993) DNA probes reveal relatedness of rice yellow dwarf mycoplasmalike organisms (MLOs) and distinguish them from other MLOs. Appl. Environ. Microbiol. 59: 1206–1212

Onuki M, Hayashi T and Sakai J (1992) Detection of antigenic proteins of mycoplasmalike organisms (MLO) associated with rice yellow dwarf from infected rice plants. Ann. Phytopathol. Soc. Jpn. 58: 613. Abstract

Ou SH (1985) Virus and MLO diseases. In: Ou SH (ed) Rice Diseases. 2nd ed. (pp 1–60). Commonwealth Mycological Institute, Surrey, United Kingdom

Shen WC and Lin CP (1994) Application of immunofluorescent staining and tissue-blotting techniques for the detection of a mycoplasmalike organism associated with sweetpotato witches' broom. Plant Pathol. Bull. 3: 79–85

Shen WC and Lin CP (1993) Production of monoclonal antibodies against a mycoplasmalike organism associated with sweetpotato witches' broom. Phytopathology 83: 671–675

Spitz M, Spitz L, Thorpe R and Eugui E (1984) Intrasplenic primary immunization for the production of monoclonal antibodies. J. Immunol. Methods 70: 39–43

Wu FY, Lin CP and Chen CC (1993) Development of cloned DNA probes for a mycoplasmalike organism associated with rice yellow dwarf. Plant Pathol. Bull. 2: 128–135