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Full Length Research Paper

Production of yam mosaic virus monoclonal antibodies in mice peritoneum

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Yam mosaic virus (YMV) is one of the most economically important virus infecting yams. Immunoassays are routinely used for laboratory diagnosis of YMV and for certification of planting materials. However, YMV antibodies, the key reagents, needed for these immunoassays are not readily available. We describe in this paper, the production of YMV monoclonal antibodies for the detection of YMV. The monoclonal antibody was produced by immunizing six weeks old BALB/c mice with YMV hybridoma cells and tapping soft peritoneal tumor tissues for antibody. Antibody titre was determined by triple antibody sandwich-enzyme-linked immunosorbent assay (TAS-ELISA) using YMV infected yam leaves and non-infected tissue culture yam leaves. The antibody produced had a titre of 1:1,310,720 and an optimal TAS-ELISA detection dilution of 1:80,000. This high-titre YMV monoclonal antibody is useful for monitoring and certification purposes.

Key words: Monoclonal antibodies, ascetic fluid, yam mosaic virus.

INTRODUCTION

Millions of people in Africa depend on yam for food each day (FAO, 2009). Rural farmers also rely on proceeds from the sales of yam tubers for the economic sustenance of their families. Yam production is however threatened by a myriad of problems such as cost and unavailability of planting materials, cost of labor, weeds, storage problems, pests and diseases, (Ezeh, 1998). Important yam pests include nematodes, beetles, termites, weevils, scale insects and rodents while diseases include those caused by fungi, bacteria and viruses (Onwueme and Charles, 1994; Hughes et al., 1997; Asare-Bediako et al., 2007). Yam viruses, which occurs both in single and mixed infections, reduce tuber yield and quality thus reducing both the economic value of the important tuber, its marketability and availability for food (Thouvenel and Dumont, 1990; Hughes et al., 1997; Odu et al., 2004; Eni et al., 2010).

Yam mosaic virus (YMV) is one of the most important economically important viruses infecting yam. YMV has been reported to be widespread in all yam producing countries around the world (Goudou-Urbino et al., 1996; Kenyon et al., 2001; Lebas, 2002; Eni et al., 2008). As yams are cultivated through vegetative planting materials (seed yam or planting sets), which accumulate viruses over time, management of YMV is best implemented through the use of certified healthy planting material. YMV certification depends heavily on the availability of sensitive and specific diagnostics. Enzyme-linked immunosorbent assay (ELISA) is a very useful tool for YMV certification. ELISA is rapid, specific and allows for several samples to be tested simultaneously (Cho, 1990). Like all immunoassays, ELISA utilizes the ability of antibodies to bind to the specific virus of interest thus antibodies remain the most critical reagent needed for ELISA.

Three antibody types commonly in use are polyclonal, monoclonal and recombinant antibodies (Peruski and Peruski, 2003). While each type of antibody has its own merits and demerits in terms of generation, cost and overall utility, monoclonal and recombinant antibodies

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have the potential of unlimited supply of uniform antibody. Monoclonal antibodies are also more specific particularly where strain differences exist (Matthews, 1991; Brattey and Burns, 1998).

Monoclonal antibodies can be produced both by *in-vitro* and *in-vivo* techniques, each with its concomitant advantages and disadvantages, however, most commercially available systems for *in-vitro* production of monoclonal antibodies, such as standard tissue culture techniques, are prohibitively expensive, requiring intensive training on cell culture techniques and the concentrations of the monoclonal antibodies produced are usually low (between 0.01 to 0.05 mg/ml) and sometimes requiring further concentration (Lang et al., 1991).

Ascetic fluid is an intraperitoneal fluid extracted from mice peritoneal tumors. High-titre monoclonal antibody (mAb) production can be accomplished by injecting commercially available mAb-producing hybridomas into the peritoneum of mice, which serves as a growth chamber for the cells. The hybridoma cells grow to high densities and continue to secrete the antibody of interest, thus, creating a high-titered (0.5 to 5.0 mg/ml) solution of monoclonal antibodies for collection. This paper reports the production of a sensitive, high titre YMV monoclonal antibody in mice peritoneum.

MATERIALS AND METHODS

Production of ascetic fluid

BALB/c mice, YMV hybridoma cells and YMV polyclonal antibodies used for this work were from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Ascetic fluid production followed the method by Jackson et al. (1999) with slight modifications. Six weeks old BALB/c mice were primed by injecting 0.3 ml of incomplete Freund's adjuvant into the peritoneum of each mouse. Two weeks after priming, rapidly growing YMV hybridoma cells in growth media were collected by centrifuging at 1,000 rpm for 10 min. The collected cells were washed once by mixing gently in phosphate buffered saline (PBS) and centrifuging for 1,000 rpm for 10 min. Two mice were injected with 5×10^6 washed hybridoma cells in 0.3 ml PBS intraperitoneally. After the injection, the mice were monitored daily for tumour formation and abdominal distension.

Soft abdominal tumours were tapped for acetic fluid collection two week after injection of hybridoma cells. The abdominal surface of each mouse was disinfected using 70% ethanol and then a 22-gauge syringe needle was inserted into the lower abdomen, dripping acetic fluid was collected into sterile BD Falcon tubes. The fluid was centrifuged at 1,000 rpm for 10 min in a microcentrifuge tube and the supernatant was carefully removed from the cell pellet and stored at -4°C.

Antibody titration

The titre of the monoclonal antibody produced was determined by triple antibody-sandwich (TAS-ELISA) as described by Thottappilly et al. (1998) with slight modifications. For enhanced antigen trapping, wells of microtitre plates were coated with 100 µl of YMV polyclonal antibodies (from the antibody bank of the Virology Unit of the IITA) diluted 1:1000 in coating buffer (0.05 M sodium carbonate buffer, pH 9.6). Following two-hour incubation at 37°C, the plates were washed three times at three minutes intervals using phosphate buffered-saline (137.00 mM NaCl, 1.46 mM KH2PO4, 7.75 mM Na2HPO4, 2.68 mM KCl, pH 7.4.) containing 0.05 % (v/v) Tween-20 (PBS-T). The wells were then blocked by incubating 200 µl of 5% skimmed milk powder (Marvel) in each well for 1 h at 37°C. Leaf sap extract (200 µl) prepared by grinding either the healthy or YMV-infected yam leaves in grinding buffer (PBS-T, 0.5 mM polyvinyl pyrrolidone (PVP)-40 and 79.4 mM Na_2SO_3) was loaded into 36 wells each and incubated overnight at 4°C. A two fold serial dilution of the produced YMV monoclonal antibody was made in PBS-T and 100 µl of each dilution was added to duplicate wells pre-coated either with healthy or infected leaf sap and the plates were incubated at 37°C for 2 h. After the washing step, 100 µl of goat anti-mouse alkaline phosphatase diluted at 1:40,000 in conjugate buffer (half strength PBS containing 0.05% (v/v) Tween-20, 0.02% (w/v) egg albumin, 0.005 mM PVP-40) was added to each well and incubated at 37°C for 2 h. Then 200 µl of ρ-nitrophenylphosphate substrate (ρNPP) (1 mg ml^{-1} in 10% diethanolamine, pH 9.8) was added into each of the wells to detect the antigen-antibody reactions.

RESULTS AND DISCUSSION

Titration of YMV ascetic fluid

BALB/c mice were used for this study to ensure histocompatibility with the YMV hybridoma cells used for this work. The mouse cell line used as hybridoma fusion partners for the production of the YMV hybridoma cells used were derived from BALB/c mice (Njukeng et al., 2002), thus, the use of a different strain of mouse may have result in the rejection of the injected cells and consequently an inability to produce the desired antibody. Successful use of inter-strain hybridomas for ascetic fluid production have however been reported, in such cases, immunedeficient or immunesuppressed mouse were used.

Two commonly used priming solutions in ascetic fluid production are pristane (2,6,10,14-tetramethyldecanoic acid) and incomplete Freund's adjuvant (Gillette, 1987). These solutions act as irritants to the mice, which respond by secreting nutrients and recruiting monocyte and lymphoid cells into the abdominal area, thus, creating a good environment for the growth of the introduced hybridoma cells. Incomplete Freund's adjuvant was used for this work because it has been suggested to have less adverse effects on the animals than pristane. Furthermore, besides causing less discomfort to the animal, incomplete Freund's adjuvant has been demonstrated to be better than pristane as a priming agent (Mueller et al., 1986; Gillette, 1987; Jones et al., 1990).

The two mice used in our work remained well during the experiments although, a marked reduction in their movement was observed as the experiment progressed. Approximately, 3 ml of ascetic fluid was collected from each mouse.

For antibody titration by TAS-ELISA, a reaction is considered to be positive if the detection factor (ratio of

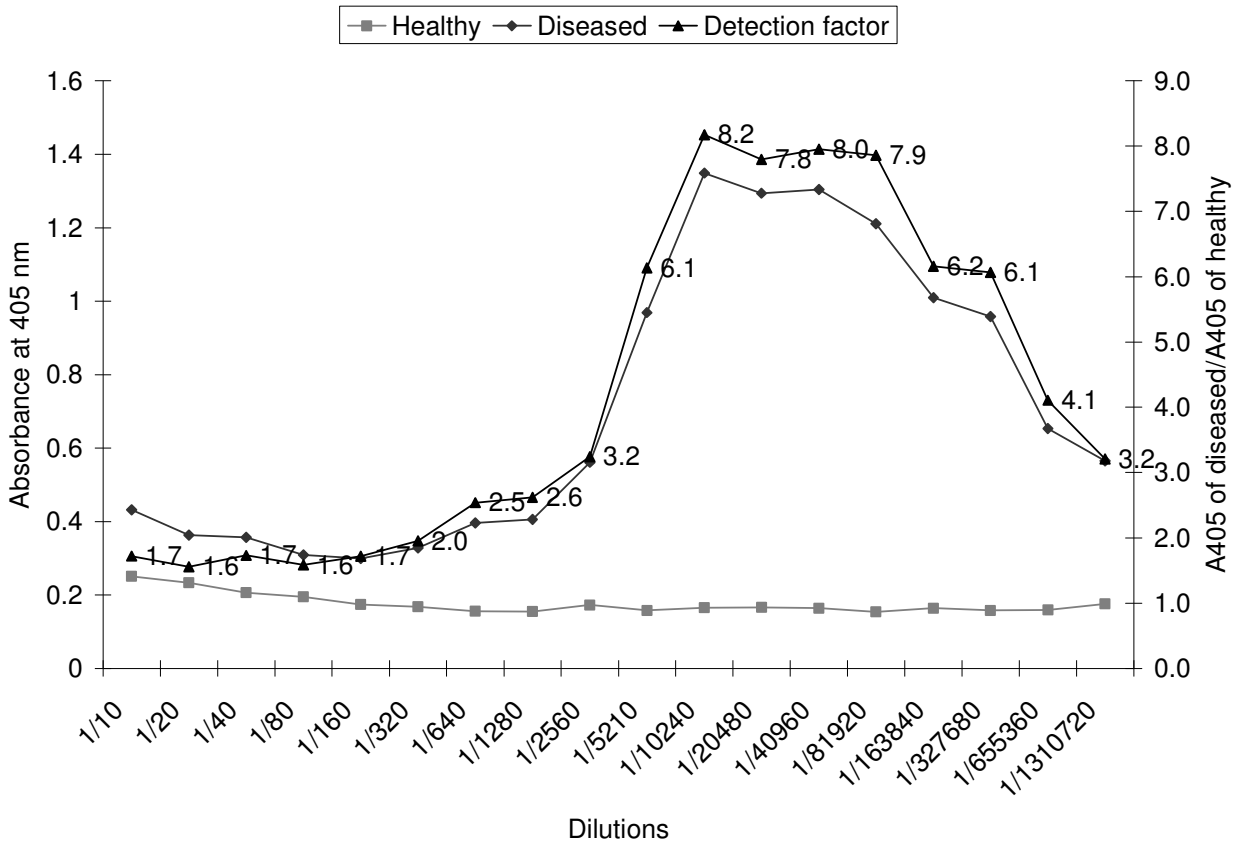

Figure 1. Detection of Yam mosaic virus (YMV) in infected and healthy yam sap by triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) using serially diluted monoclonal antibody. A_{405} of infected sap/A_{405} of healthy sap ratio > 2 is considered to be positive.

the mean absorbance value of the infected sap and the healthy sap) at that dilution is greater than 2 (Thottappilly et al., 1998).

$$\text{Detection factor} = \frac{\text{Mean } A_{405} \text{ of infected sap}}{\text{Mean } A_{405} \text{ of healthy sap}}$$

Positive reactions were observed from 1:640 dilutions with a detection factor of 2 and peaked at 1:10,240 with a detection factor of 8.2. Subsequent monoclonal antibody dilutions detected YMV in infected sap but, with declining detection factors and detection continued to the last antibody dilution of 1:1,310,720 (Figure 1). Noting that the detection factor remained very close to the peak between 1:10,240 and 1:81,920 dilutions (8.2 to 7.9), the optimal TAS-ELISA working dilution for this antibody can be stretched as far as 1:80,000.

Although, the volume of monoclonal antibody produced was low, this is adequately compensated for by the high titre of the antibody. The optimal TAS-ELISA working dilution of most available *in-vitro* produced YMV monoclonal antibodies 1:500 which is way lower than 1:80,000 obtained for the current antibody. This high titre is also economically significant particularly considering the cost and time that would be required to produce a similar total TAS-ELISA ready-to-use dilution of YMV monoclonal antibody *in-vitro*.

The YMV monoclonal antibody produced in this study would boost current efforts to reduce the spread of YMV through enhanced monitoring and certification. Such monitoring and certification activities are continuously ongoing at the International Institute of Tropical Agriculture (IITA) and other international and National Agricultural Research Institutes/Stations worldwide, most of which depends on IITA for YMV antibody supply, yam being one of the IITA mandate crops.

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