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*Full Length Research Paper*

# Production of monoclonal and polyclonal antibodies against a Nigerian isolate of banana streak virus

**Agindotan B.O.[1,2,*], Thottappilly G.[2,λ], Uwaifo A.[1] and Winter S.[3]**

[1]Biochemistry Department, University of Ibadan, Ibadan, Nigeria
[2]International Institute of Tropical Agriculture, Ibadan, Nigeria.
[3]DSM-Deutsche Sammlung von Mikroorganismem und Zellkulturen Abt. Pflanzenviren, Braunschweig, Federal Republic of Germany

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**Banana streak virus is serologically and genomically heterogenous worldwide and there has been the need to produce antibodies that can detect all known serotypes of this virus. Antibody production requires purified virus, since BSV titre is low in *Musa* tissues, there was the need for an efficient method of purifying the virus. We are reporting the first production of two monoclonal antibodies, BSV 3F9/1 and BSV 3D4/2, against an isolate of BSV. Culture fluids of BSV 3F9/1 and BSV 3D4/2 had antibody titres of 1:204,000 and 1:6400, respectively by ELISA. The two monoclonal antibodies detected all isolates of BSV that were detected by the homologous mouse polyclonal antibodies. Caesium gradient centrifugation reduced yield of BSV during purification. We described a method of purification, which excluded the caesium gradient step and yet increased BSV yield by about 15-fold. The virus preparation obtained by this new method was used to produce BSV-specific mouse and rabbit polyclonal antibodies. These BSV monoclonal antibodies together with the polyclonal antibodies were used for the detection of BSV.**

**Key words:** Banana streak virus, monoclonal antibodies, polyclonal antibodies, *Musa*, TAS-ELISA, polysynthetic antibodies, antigen-coated dot ELISA.

## INTRODUCTION

Plantain and banana (*Musa* spp.) are grown in about 121 countries (F.A.O., 2001) providing a major source of carbohydrates for over 400 million people in tropical countries (Swennen et al., 1995). Over 102 million metric tonnes are produced yearly (F.A.O., 2001). Plantain and banana also provide a major source of income for smallholders (Nweke et al., 1988).

*Banana streak virus* (BSV), genus *Badnavirus*, the causal agent of viral leaf streak, is considered to be the most frequently occurring virus of *Musa* worldwide, and has been associated with yield loss (Lockhart et al., 1998; Davis et al., 2000; Daniells et al., 2001). The occurrence of BSV in a significant proportion of the improved tetraploid *Musa* hybrids has been reported (Lockhart et al., 1998).

Difficulties in obtaining virus-free plantlets through tissue-culture techniques (Daniells et al., 1995; Hughes, 1998; Helliot et al., 2002) have caused major setbacks on the distribution of high quality *Musa* cultivars. Moreover, the integration of the virus genome into the host genome (Dallot et al., 2001; Geering et al., 2001; Harper et al., 2002) has been making exclusive nucleic acid-based detection of the virus frustrating, as virtually

*Corresponding Author; Present address: Plant, Soil and Entomological Sciences Dept., University of Idaho, Moscow, ID 83844, USA, e-mail: abright@uidaho.edu, fax: +1-208:8857760, tel: +1-208-885-5827.

[λ]Present address: Mahyco Research Foundation, Kamalapuri colony, Hyderabad- 500073, India.

all *Musa* tissues test positive to BSV.

FAO/IPGRI Technical Guidelines for the Safe International Movement of *Musa* Germplasm (Diekmann and Putter, 1996) demands the screening of *Musa* plantlets for the episomal form of BSV before they can be transported from one country to another. Immunosorbent electron microscopy (ISEM), enzyme linked immunosorbent assay (ELISA) and Immuno-capture PCR (Harper et al., 1999) that can specifically discriminate between episomal (the particulate form of the virus) and non-episomal virus require readily available source of BSV-specific antibodies. BSV-specific polyclonal antibodies were not readily available due to the difficulties in obtaining infected leaf tissues because of the periodicity of symptom expression (which is correlated with the presence of the virus), absence of propagation hosts, low titre of the virus in infected host, and the difficulties in purifying the virus. (Ndowora and Lockhart, 1996; Thottappilly et al., 1998).

Before this project, BSV-specific monoclonal antibodies had not been reportedly produced, and the BSV-specific, good quality polyclonal antibodies were not readily available. Because of the genetic and serological diversity of BSV worldwide (Geering et al, 2000; Lockhart and Olszewski, 1993), there has been the need to produce synthetic polyclonal antibodies from monoclonal antibodies raised against different serotypes of BSV. In this paper we describe the production of first BSV-specific monoclonal antibodies against the virus, and showed that they detected as many BSV isolates as the homologous polyclonal antibodies.

## MATERIALS AND METHODS

### *Musa* samples

Leaves samples of *Musa* cultivars from the International institute of Agriculture (IITA) stations at Ibadan and Onne, both in Nigeria, were harvested, stored at $4^0$C and tested for BSV within three days of harvest.

### Initial Polyclonal antibodies

The initial BSV mouse and rabbit antisera were obtained from Prof. B.E.L. Lockhart, University of Minnesota, USA; and IITA.

### Purification of BSV

BSV was purified from BSV-infected leaf tissues of a hybrid plantain, TMPx 7002-1 obtained from IITA, Onne, Nigeria. Two methods were used for BSV purification. One method (Method A) included sucrose cushion centrifugation step, while the other caesium gradient centrifugation step (Method B). Both methods were modified versions of Thottappilly et al. (1998).

**Method A:** Fresh 200 g BSV-infected leaf tissues of TMPx 7002-1 (excluding midribs) were cut into pieces and ground into powder in liquid Nitrogen and blended in 200 mM phosphate buffer, pH 6.0, containing 1 % (w/v) sodium sulphite and 2 % (w/v) polyvinyl pyrrollidone in ratio 1:3 (w/v). The extract was filtered through two layers of cheese clothe and centrifuged at 12 000 *g* for 20 min, to remove plant materials. To the filterate, 6% (w/v) polyethyleneglycol (p-40000) and 1.5% (w/w) NaCl, were added, stirred at $4^0$C for 1 h, and then centrifuged at 12000 g for 20 min. The pellet obtained was suspended in 100 mM sodium phosphate buffer, pH 7.2, containing 1.5% (w/w) sodium chloride. Triton-X 100 was added to the suspension at 0.5% (v/v), and stirred for 1 h at $4^0$C, followed by centrifugation at 12 000 *g* for 20 min. The supernatant was subjected to 30% (w/v) sucrose cushion centrifugation at 105, 000 g for 2 h in a Beckman Type 35 rotor. The virus pellet was re-suspended in the suspension buffer, equal volume of cold chloroform added, stirred for 20 min at $4^0$C, and then centrifuged at 12000 g for 10 min at $4^0$C. The aqueous phase was carefully separated and then centrifuged at 12 000 g for 5 min. The supernatant obtained, hereafter referred to as BSV Prep A, was examined for BSV by ISEM using rabbit antiserum obtained from Prof. B.E.L. Lockhart.

**Method B:** Starting with fresh 200g BSV-infected leaf tissues of TMPx 7002-1, the steps of purification were the same as in method A up to the point before sucrose cushion centrifugation. Sucrose cushion centrifugation was replaced with caesium gradient centrifugation. The virus suspension, 1 ml per tube, was layered on preformed caesium sulphate step gradient. The gradient was performed by filling each Beckman ultra-clear centrifuge tubes (14 x 89 mm) with 1ml 10%, 2.5 ml 15%, and 3 ml 30% (w/v) $Cs_2SO_4$, in 10 mM Tris-HCl, pH 7.4 buffer containing 10% (w/v) sucrose. Caesium gradient centrifugation was done at 105 000 g for 6 h in a SW 41 rotor. Light scattering bands were colleted separately and dialysed overnight in suspension buffer. The collected bands were referred to as BSV preparation Bii (lower band) and B (upper band). The virus preparations were sent for electron microscopic examination for BSV. They were also tested for BSV by TAS-ELISA, using the rabbit antibodies obtained from Prof. B.E.L. Lockhart and the mouse polyclonal antibodies previously produced in IITA.

### Production of BSV mouse polyclonal antibodies

Four 3-week old Balb/c mice each injected sub-cutaneously with 200 µl virus preparation A emulsion which consisted 500 µl Freund's complete Adjuvant + 100 µl BSV prep A (equivalent to 5 g of BSV-infected leaf tissue) + 400 µl sterile water. Another set of four mice was each injected sub-cutaneously with 200 µl virus prep B emulsion which consisted 500 µl of BSV prep B (equivalent to 125 g of BSV-infected leaf tissue) + 500 µl of Freund's complete adjuvant. Two weeks after, the immunisation was repeated but with Freund's incomplete Adjuvant replacing the complete adjuvant. A month after the first immunization, the mice were boosted with the same quantities of virus preparation but with no adjuvant. Three days after the third immunization, the mice were sacrificed and blood obtained by heart puncture. The blood obtained from each set of the sacrificed mice was pooled, allowed to clot and antiserum obtained (Ball et al., 1990). The pooled antiserum from the set of mice immunized with BSV preparation A was referred to as BSV mouse antiserum A, while that obtained from mice immunized with BSV preparation B was referred to as BSV mouse antiserum B.

**Note:** The exact concentration of purified virus could not be determined since the absorption coefficient of BSV is unknown.

### BSV rabbit polyclonal antibody production

A 3-month old rabbit was immunised intramuscularly with a mixture of 500 µl of BSV prep A and 500 µl of Complete Freund's Adjuvant

emulsion. The immunisation was repeated two more times with Incomplete Freund's adjuvant replacing Complete Freund's adjuvant. Five days after the third immunisation, blood was obtained from the ear of the rabbit and processed for antiserum and antibody purified through 1 ml HiTrap protein G-II kit (Sigma), following the manufacturer's protocol.

#### Hybridoma production

**Immunization:** Monoclonal antibody production was as described by Powell (1990). Four 3-week old Balb/c mice were each immunized with BSV preparation A as previously described for BSV mouse polyclonal antibody production, except that a week after the second immunization, tail bleed test was done. Two spleens of the immunized mice were used for two different fusion experiments. Also, four other 3-week old mice were also sacrificed and their thymus cells squeezed out and used as feeder cells; two thymuses per fusion.

**Fusion:** The two fusions were done separately using FOX-NY and X63/Ag8.653 myeloma cell lines as fusion partners. The hybridoma cell culture fluids were screened for BSV specific antibody by TAS-ELISA. BSV-infected and BSV-negative TMPx 7002-1 leaf sap at 1:5 dilution in BSV grinding buffer (PBS-T + 2% w/v polyvinyl chloride (PVP-40000) + 1% w/v sodium sulphite) were used as positive and negative controls respectively. The antibody titres of the antisera, obtained from the mice whose spleens were used for the fusion, were determined by TAS-ELISA.

#### TAS-ELISA

The TAS-ELISA procedure was as described by Thottappilly et al (1998), except that BSV rabbit IgG at a concentration of 2 µg/ml in coating buffer was used to coat microtiter plates (Dynatech). Healthy and infected plantain hybrid TMPx 7002-1 leaf sap at 1 in 5 dilution in grinding buffer (PBS-T + 2% w/v polyvinyl chloride (PVP-40000) + 1% w/v sodium sulphite) were used for screening the culture fluid for BSV-specific antibodies.
Mean absorbance values at 405 nm that were twice or more than the mean of the healthy samples and were greater than 0.100 were considered to be BSV-infected samples (Thottappilly et al., 1998).

#### Monoclonal antibody titre and Isotyping

The titres of antibodies in the culture fluids were determined by TAS-ELISA. Isotyping was done using Sigma mouse monoclonal antibody isotyping reagents stock No. ISO-2 and capture ELISA procedure as described in Sigma isotyping kit manual.

#### Screening of *Musa* leaf samples

*Musa* leaf samples collected from IITA stations in Onne and Ibadan were tested for BSV. The TAS-ELISA protocol earlier described was used except that the culture fluids of the two BSV monoclonal antibodies (BSV 3F9/1, at 1/500 dilution, and BSV 3D4/2, at 1/20 dilution), and BSV mouse antiserum (at 1/30,000 dilution) were used separately as detecting antibodies.

#### Sensitivity determination

The sensitivities of the monoclonal antibodies for BSV detection by TAS-ELISA and antigen-coated dot-ELISA were determined by sap dilution end-point of a BSV-infected Tropical *Musa* plantain hybrid (TMPx 7002-1). BSV- infected and healthy plantain leaf tissue of TMPx 7002-1, 1 g each, was ground in 4 ml of BSV extraction buffer (PBS, 2% (w/v) PVP and 1% (w/v) $Na_2SO_3$). The 1:5 diluted leaf extracts were further diluted in a three-fold series from 1:15 to1: 18,175, and tested for the presence of BSV by these two techniques.

For TAS-ELISA, BSV rabbit IgG at a concentration of 2 µg/ml was used for coating the wells of the microtitre plates. Serially diluted healthy and infected leaf sap extracts added. The monoclonal antibody BSV 3F9/1 at 1:250 dilution in PBS, was used as secondary antibody. Other steps were as earlier described in the TAS-ELISA.

Antigen-coated dot-ELISA as described by Banttarri and Godwin (1985) was followed except that Optitran BA-S 85 reinforced nitrocellulose membrane, 0.45 µm (Schleicher & Schwell), and the clarification step was as follows. To 0.5 ml of each serially diluted healthy and infected leaf sap extracts, 3 drops of Triton-X 100 were added and the mixture vortexed for 2 min. It was followed by the addition of equal volume (0.5 ml) of chloroform and the mixture emulsified by stirring in the cold room. The emulsified mixture was separated into two phases by centrifugation at 10000 *g* for 10 min. The aqueous phase was decanted and referred to as chloroform-clarified sap. The chloroform-clarified and the crude sap extracts of the different serial dilutions were dotted on nitrocellulose membranes at 5 µl per sample and then allowed to air-dry. Monoclonal antibody culture fluid (BSV 3F9/1), at 1:125 dilution in PBS was used as detecting antibody.

## RESULTS

### BSV purification

With method B, two close bands were obtained at about 1.0 cm and 1.5 cm from the bottom of centrifuge tubes. The lower band was dirty. Both bands tested positive to BSV by TAS-ELISA, but the upper, cleaner band gave a higher reading suggesting there were more BSV particles in it (ELISA readings: lower band = 0.385, upper band = 0.450) (Table 1). The upper band was referred to as BSV preparation B. The lower band was subjected to another caesium gradient centrifugation, for further purification of the band. Unfortunately, no virus band was visible under light scattering, except a precipitate. Inferring from the volume of the virus preparations and ELISA or ISEM scores, method A yielded 15 times more virus than method B (Figure 1 and Table 1).

### BSV polyclonal antibodies

The antibody titres of the pooled mouse antiserum raised against BSV preparations A and B were 1 in 256000 and 1 in 64000, respectively, after 1 hr of substrate incubation, in TAS-ELISA). The ELISA readings of the "healthy" (BSV-free) samples remained less than 0.1, after 1hr. of substrate incubation at antiserum dilution of less than 1 in 16000 (Figure 2).

The first bleed rabbit antiserum produced against BSV preparation A when used as coating antibody in TAS-ELISA, trapped BSV at the lowest antiserum dilution of 1 in 102400. About 10 mg of IgG was obtained from 3

**Table 1.** Comparison of yield and the biophysical properties of BSV preparations.

| Virus Purification Indices | BSV Preparation A | BSV Preparation B |
|---|---|---|
| Mass of leaf tissue | 1 Kg | 1Kg |
| Volume of BSV preparation | 20 ml | 4 ml |
| Absorbance at 260 nm | 1.433 ± 0.005 | 0.572 ± 0.004 |
| Absorbance at 280 nm | 1.084 ± 0.006 | 0.480 ± 0.004 |
| Absorbance 260 nm/280 nm | 1.32 ± 0.005 | 1.19 ± 0.004 |
| ISEM score (BSV per screen) | 12 ± 0.4 | 4 ± 0.3 |
| ELISA score (at 405 nm) | 1.276 ± 0.020 | 0.45 ± 0.017 |
| Inferred relative yield of BSV | 15 | 1 |

[a]The 15-fold yield of BSV preparation A over BSV preparation B was deduced from the 5-fold volume yield and a 3-fold concentration (from the ELISA and ISEM results)

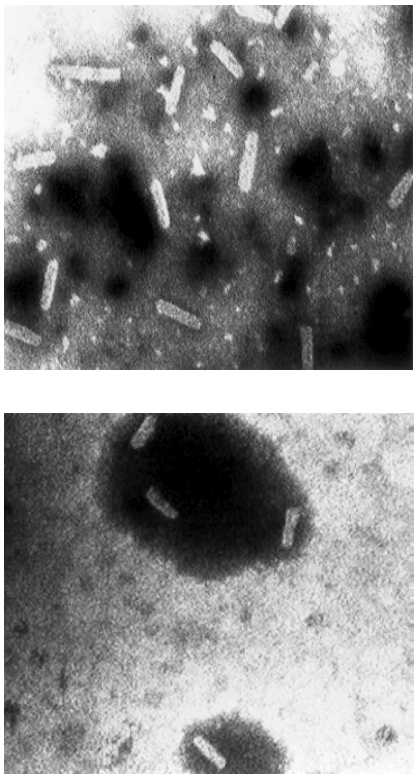


**Figure 1.** Immunosorbent Electron Micrographs of BSV Preparations. A = ISEM of preparation A; B = ISEM of preparation B. Magnification is x 22000. Antibody incubation was for 15 min at 25$^0$C and virus incubation at 25$^0$C for 1 h.

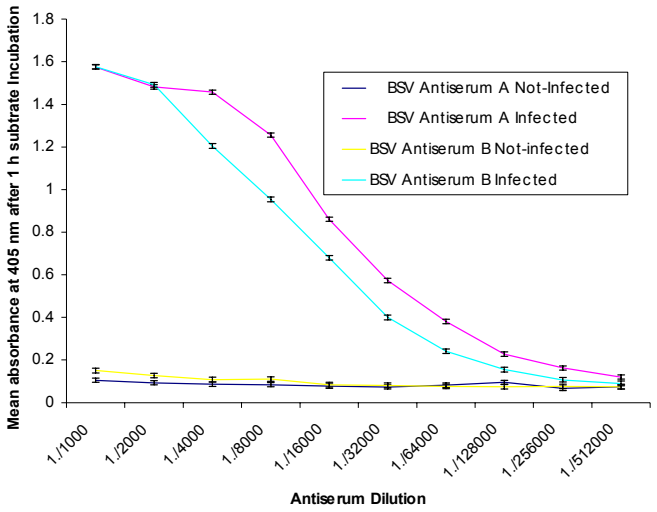


**Figure 2.** Comparing BSV preparations A and B mouse antisera for the detection of BSV in TAS-ELISA.
Mouse antisera against BSV preparations A and B were diluted in two-fold series. They were screened against 1 in 5 dilution of healthy (non-infected) and BSV-infected banana leaf sap. Substrate incubation was 1h at 37$^0$C. The antibody titre of each of the antisera was its least dilution that gave positive test for BSV. Substrate incubation was for 1h at 37$^0$C. All tests were done in triplicates.

ml of the antiseum. At coating concentration of about 0.5 µg/ml of the BSV rabbit IgG, the absorbance at 405 nm was above 1.0 for the infected sample (Figure 3).

## BSV monoclonal antibody production

Only two hybridoma cell lines were obtained secreting BSV-specific antibodies: one cell line per fusion (Table 2). Each cell line produced one clone of BSV monoclonal antibody secreting hybridoma cells. That is, only one BSV-specific monoclonal antibody was obtained per fusion. They were coded BSV 3F9/1 and BSV 3D4/2. BSV 3F9/1 hybridoma was obtained by fusing FOX-NY myeloma cell with B-lymphocyte, while BSV 3D4/2 hybridoma by fusing X-63/Ag 8.653 myeloma cell with B-lymphocyte.

The antibody titres of the antisera of the two mice whose spleens were used for fusion were 1:256,000 and 1:128,000. The antisera obtained from these mice were apparently clean because the ELISA score against

**Table 2.** Screening of cultures of hybridoma cell lines for BSV antibody secretion.

| Fusion | Fraction of Wells with hybridomas | Fraction of wells with host antibodies | Fraction of wells with BSV antibodies | Fraction with Immunoglobulins of unknown antigen specificity |
|---|---|---|---|---|
| 1st Fusion | 476/480 (99%) | 31/476 (15%) | 1/476(0.002%) | 444/476 (93%) |
| 2nd Fusion | 465/480 (97%) | 42/465 (11%) | 1/465 (0.002%) | 422/465 (90%) |

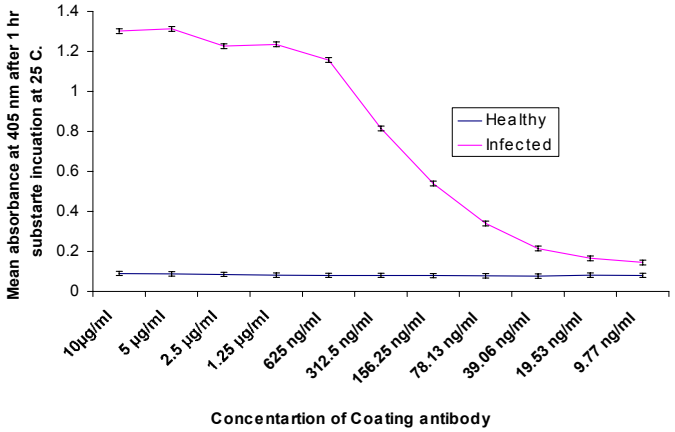


**Figure 3.** BSV rabbit polyclonal antibodies as coating antibodies in TAS-ELISA.
BSV rabbit IgG of 10 µg/ml 2-fold serial dilutions tested for antibody trapping ability. Healthy (non-infected) and BSV-infected banana leaf extracts were used at 1 in 5 dilution. Substrate incubation was for 1h at 37$^0$C.

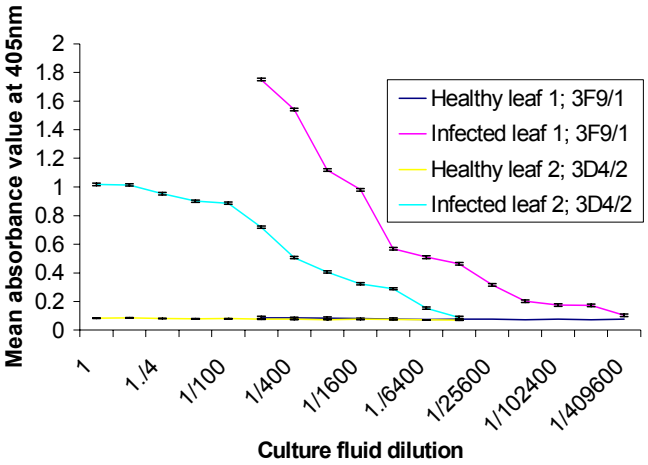


**Fig 4.** BSV antibody titres of BSV 3F9/1 and BSV 3D4/2 monoclonal antibody Culture fluids.
Two-fold serial dilutions of two monoclonal antibodies culture fluids. For Monoclonal antibody culture fluid BSV 3D/4, the starting concentration was the undiluted culture, while that of culture fluid BSV 3F9/1 was 1 in 200. Healthy (non-infected) and BSV-infected plantain leaf extracts were used at 1 in 5 dilution. Substrate incubation was for 1h at 37$^0$C. The antibody titre of each of the culture fluids was its least dilution that gave positive test for BSV. Substrate incubation was for 1h at 37$^0$C. All tests were done in triplicates.

healthy samples were less than 0.1, after 1 h of substrate incubation (results not shown).

## Monoclonal antibodies specificity

The two monoclonal antibodies detected BSV in 20 of 28 *Musa* clones. Out of the 8 clones with asymptomatic leaves, 3 tested positive to BSV. Of the 20 with sympomatic leaves, 17 were positive for BSV (Table 3). The monoclonal antibodies detected BSV in the same *Musa* leaf samples as the homologous mouse polyclonal antibodies.

## Monoclonal antibodies titres and isotyping

The antibody titres of the monoclonal antibodies culture fluids were determined by TAS-ELISA; 1 in 204, 800 and 1 in 6,400 for BSV 3F9/1 and BSV 3D4/2, respectively (Figure 4). Their immunoglobulin type was IgG 2a.

## Sap dilution end-point determination

The comparative BSV-infected leaf sap dilution end-points of TAS-ELISA and antigen coated dot-ELISA were 1:1215 when crude sap was used. However, when chloroform clarified sap was used, the sap dilution end-point was 1:135 (Figure 5).

## DISCUSSION

Purification method A using sucrose cushion centrifugation yielded 15-times more BSV than method B that included caesium gradient. The reason is most likely that the caesium gradient centrifugation destroyed some of the virus particles. The absence of a band after second caesium centrifugation of the lower band tends to support this argument. Although it is well known that high purification is always at the expense of yield; for antibody production, the yield loss can only be justified if the inclusion of a purification step, will lead to a virus preparation that can be used to produce a more virus-specific, “cleaner” antiserum. However, this was not the case, as the antisera produced against the purified BSV

**Table 3.** Screening of *Musa* clones for BSV with monoclonal and polyclonal antibodies by TAS-ELISA.

| *Musa* Clone | Symptom | [a]Mean absorbance value (n=3) at 405 nm at 1h substrate incubation time. [b]m.s.e. = ± 0.015 | | |
|---|---|---|---|---|
| | | BSV 3F9/1 Monoclonal Antibody | BSV 3D4/2 Monoclonal Antibody | BSV Mouse antiserum |
| FHIA 1 | Symptomless | 0.073 | 0.065 | 0.091 |
| FHIA 2 | Symptomless | [c]**0.333** | **0.199** | **0.287** |
| FHIA 3 | Symptomless | **0.483** | **0.137** | **0.315** |
| SH 3363 | Yellow streak | 0.077 | 0.066 | 0.08 |
| SH 3436 | Symptomless | 0.071 | 0.069 | 0.087 |
| SH 3640 | Yellow streaks | **0.99** | **0.294** | **0.501** |
| TMPx 548-4 | Yellow streaks | **1.337** | **0.423** | **0.983** |
| TMPx 548-9 | Yellow streaks | **0.438** | **0.194** | **0.4** |
| TMPx 548-9 | Yellow streaks | **1.696** | **0.655** | **1.479** |
| TMPx 582-4 | Yellow streaks | **0.592** | **0.235** | **0.493** |
| TMPx 1112-1 | Yellow streaks | **1.18** | **0.335** | **0.892** |
| TMPx 1658-4 | Yellow streaks | **1.939** | **0.831** | **1.236** |
| TMPx 26378 | Yellow streaks | **0.654** | **0.364** | **0.615** |
| TMPx 2796-5 | Yellow streaks | **0.519** | **0.233** | **0.479** |
| TMPx 4698-1 | Symptomless | 0.068 | 0.086 | 0.07 |
| TMPx 4698-1 | Yellow streaks | **0.768** | **0.21** | **0.659** |
| TMPx 5511-2 | Yellow streaks | **1.24** | **0.339** | **0.938** |
| TMPx 5511-2 | Symptomless | 0.066 | 0.067 | 0.089 |
| TMPx 7002-1 | Yellow streaks | **1.123** | **0.299** | **0.705** |
| TMPx 7152-2 | Symptomless | **0.44** | **0.163** | **0.37** |
| TM 3x 26388 | Yellow streaks | **0.782** | **0.306** | **0.725** |
| TM 3x 26388 | Yellow streaks | **0.81** | **0.246** | **0.801** |
| TM 3x 26636 No 2 | Yellow streaks | 0.084 | 0.072 | 0.088 |
| TMB2x 5105-1 | Yellow streaks | **0.412** | **0.196** | **0.396** |
| Cardaba | Yellow streak | 0.068 | 0.061 | 0.071 |
| Lakanau | Yellow streaks | 0.077 | 0.072 | 0.08 |
| BSV infected plantain | Yellow streaks | **1.876** | **0.542** | **1.251** |
| Healthy plantain | Symptomless | 0.061 | 0.06 | 0.062 |

[a]Mean ELISA reading that was at least twice that of healthy plantain leaf (0.062) was indicative of the presence of BSV, and as such considered as a positive test for BSV.
[b]m.s.e.= Mean standard error.
[c]The bold ELISA readings were those scored positive for BSV.

obtained by these two methods were very good. They were BSV specific, of high BSV titre and "clean". In fact, method A virus preparation produced antiserum of higher BSV antibody titre. Consequently, for the purpose of antiserum production, the method A of BSV purification is a better choice.

In spite of two fusions, high fusion efficiency (97-99% of the wells had hybridoma cells) and the production of BSV-specific antisera by the immunized mice whose spleens were used for the fusions, only two hybridoma lines produced BSV-specific monoclonal antibodies. Close to zero fusion specific efficiency had been

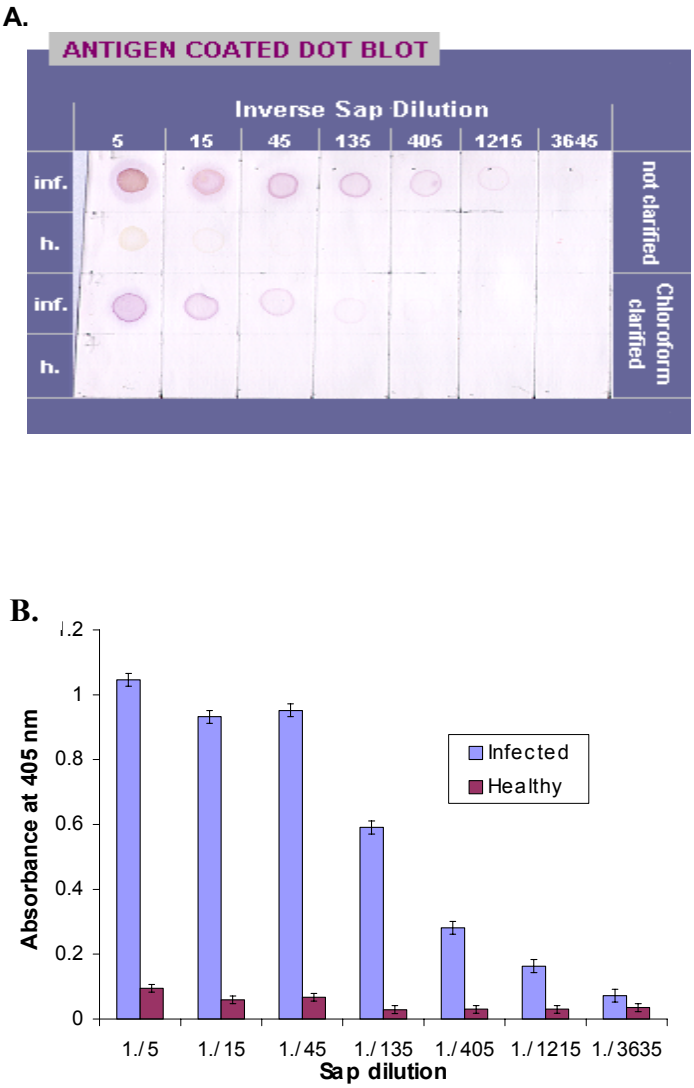


**Figure 5.** Sap dilution end-point determination of BSV-infected leaf sap by antigen-coated Dot-ELISA and TAS-ELISA.
**A**= Antigen coated Dot-ELISA. Spots observed in the 'healthy' row of 'not clarified' sap were coloration from the leaf sap pigment. The sap dilution end-point is the least dilution of sap that could be detected without false positive signal from the healthy sap.
**B**= TAS-ELISA. The sap dilution end-point was the least leaf sap dilution that was detected. That is, the least dilution which gave ELISA reading that was at least twice that of the 'healthy'. Substrate incubation was for 1h at 37$^0$C. All tests were done in triplicates.

attributed to inadequate stimulation of antigen-specific B-lymphocytes in *in vivo* immunization (Jordan, 1990). Since the spleen donor mice for the fusion had high titre of BSV-specific antibody, so close to zero fusion specific efficiency could not have arisen due to the reason adduced above. It could be that the sucrose used for a stage in the virus purification bound to the macromolecules that served as haptens and therefore caused them to be highly immunogenic. This could have stimulated higher immune response (more sucrose-specific B-lymphocytes stimulated) than BSV itself. A large number of the hybridomas cell lines secreting immunoglobulins of unknown antigen-specificity could have be in fact sucrose-specific antibody secreting hybridomas.

The two monoclonal antibodies detected BSV in different asymptomatic (4 of 11) and symptomatic (22 of 29) clones of *Musa* stocked in IITA, Nigeria. IITA *Musa* clones are propagated vegetatively by tissue culture techniques and through suckers (Hughes, 1998). So, it is probable that the same type serotype of BSV could have been propagated.

The seven symptomatic *Musa* leaf samples that tested negative to BSV could have been infected with a CMV, which also produces chlorotic streaks that may be similar to those caused by BSV (Lockhart, 1994). Another possibility is that these leaves were infected with a serotype of BSV not detectable by our monoclonal and polyclonal antibodies. Geering et al. (2000) had reported incidence of genetic diversity of BSV in a single country (Australia). Genetic diversity could also lead to serotype diversity.

TAS-ELISA and antigen-coated dot-ELISA had the same sensitivity of detection of BSV infected leaf sap when the monoclonal antibody, BSV 3F9/1 was used as a detecting antibody. The clarification of sap needed by the dot-ELISA for clarity of result reduced the sensitivity of this technique to 1: 145, below that of TAS-ELISA. Jordan and Hammond (1988) had also observed reduction in sap dilution end-point of chloroform clarified *bean mosaic yellow virus* (BYMV) infected *Nicotiana benthamiana* leaf sap. Despite the reduction of sensitivity after sap clarification, antigen-coated dot-ELISA could be used for a wholly monoclonal antibody-based detection method for BSV.

These monoclonal antibodies along side with those that may be produced by other workers could be pooled together for broad-spectrum detection of known BSV serotypes.

## ACKNOWLEDGEMENT

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### REFERENCES

Bantarri EE, Godwin PH (1985). Detection of potato viruses S, X and Y by enzyme-linked immunosorbent assay on nitrocellulose membrane (Dot ELISA). Plant Dis. 69:202-205.

Dallot S, Acuna P, Rivera C, Ramirez P, Cote F, Lockhart BEL, Caruana, ML (2001). Evidence that the proliferation stage of micropropagation procedure is determinant in the expression of *Banana streak virus* integrated into the genome of the FHIA 21 hybrid (*Musa* AAAB). Arch. Virol. 146: 2179-2190.

Daniells J, Thomas JE, Smith BJ (1995). Seed transmission of *Banana streak Badnavirus* confirmed. InfoMusa 4:7.

Daniells JW, Geering AD, Bryde NJ, Thomas JE (2001). The effect of banana streak virus on the growth and yield of dessert bananas in tropical Australia. Annals of Applied Biology 139: 51-60.

Davis RI, Geering ADW, Thomas JE, Guna TG, Rahamma S (2000). First records of *Banana streak virus* on the Island of New Guinea. Australasian Plant Pathol. 29: 281.

Diekmann M, Putter CAJ. (1996). FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm. 2nd editiion. Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome.

Food and Agricultural Organization (FAO) (2001). FAO Year Book, Production 2000, FAO, Rome Italy.

Geering ADW, McMichael LA, Dietzgen RG, Lockhart BEL (2001). Analysis of the distribution and structure of integrated Banana streak virus DNA in a range of *Musa* cultivars. Mol. Plant Pathol. 2: 207-213.

Geering ADW, McMichael LA, Dietzgen RG, Thomas JE (2000). Genetic diversity among *Banana streak virus* isolates from Australia. Phytopathology 90, 921-927.

Hammond M, Jordan RL (1990). Dot blots (viruses) and colony screening. In serological methods for the Detection and Identification of Viral and bacterial Plant Pathogens. A Laboratory Manual. Hamptom R, Ball S, De Boer S (Editors). APS press. 389pp.

Harper G, Dahal G, Thottappilly G, Hull R (1999). Detection of episomal *Banana streak badnavirus* by IC-PCR. J. Virol. Methods 79: 1-8.

Harper G, Hull R, Lockhart BEL, Olszewski N (2002). Viral sequences integrated into plant genomes. Annu. Rev. Phytopathol. 40: 119-136.

Helliot B, Panis B, Poumay Y, Swennen R, Lepoivre P, Frison E (2002). Cryopreservation for the elimination of *Cucumber mosaic* and *Banana streak viruses* from banana (*Musa* spp.). Plant Cell Rep. 20: 1117-1122.

Hughes Jd'A (1998). *Banana streak badnavirus* research at the International Institute of Tropical Agriculture.. In: *Banana Streak Virus: A Unique Virus-Musa Interaction?* (Frison EA, Sharrock S, editors). Proceedings of a workshop of the PRO*MUSA* Virology working group held in Montpellier, France, 19-21 January 1998.

Jordan RL (1990). Strategy and techniques for the production of monoclonal antibodies; monoclonal antibody applications for viruses. In Serological methods for the detection and identification of viral and bacterial plant pathogens. A Laboratory Manual. Hamptom R, Ball S, De Boer S. (Editors). APS press. 389 p.

Jordan RL, Hammond J (1988). Epitope specificity of strain-, virus-, subgroup-specific and potyvirus group cross-reactive monoclonal antibodies (Abstract). Phytopathology 78: 1600.

Lockhart BEL (1986). Purification and serology of a bacilliform virus associated with banana streak disease. Pythopathology 76: 995-999.

Lockhart BEL (1994). Banana streak virus. In Compendium of Tropical Diseases (Ploctz, et al., eds). St Paul MN, USA: APS Press 88pp.

Lockhart BEL, Olszewski NE (1993). Serological and genomic heterogeneity of *Banana streak badnavirus*: implications for virus detection in *Musa* germplasm. In breeding banana an plantain for resistance to diseases and pests, pp. 105-113. Edited by Ganry J. Montpellier:CIRAD, in collaboration with INIBAP (International Network for the Improvement of Banana and Plantain.

Lockhart BEL, Ndowora TC, Olszewski NE, Dahal G (1998). Studies on integration of *Banana streak badnavirus* sequences in *Musa*: Identification of episomally-expressible badnaviral integrants in *Musa* genotypes. In: *Banana Streak Virus: A Unique Virus-Musa Interaction*? (Frison EA and Sharrock SE, editors). Proceedings of a workshop of the PRO*MUSA* Virology working group held in Montpellier, France, 19-21 January 1998.

Ndowora TCR, Lockhart BEL (1996). Improved serological methods for detecting *Banana streak virus*. InfoMusa 10:37.

Nweke F, Njoku J, Wilson GF (1988). Productivity and limitations of plantain (*Musa* spp. cv AAB) production in compound gardens in South-eastern Nigeria. Fruits 43: 161-166.

Powell CA (1990). Detection of Tomato Ringspot Virus with Monoclonal antibodies. Plant Disease 74: 904-90.

Swennen R, Vuylsteke D, Oritz R (1995). A Global Patnership (Jones DR editor) pp. 130-156. INIBAP, Montellier, France.

Thottappilly G, Dahal G, Lockhart BEL (1998). Studies on a Nigerian isolate of *Banana streak badnavirus* I. Purification and enzyme-linked immunoassay. Ann. Appl. Biol. 132:253-261.