Enterovirus Recovery with Vegetable Floc

JACK KONOWALCHUK AND JOAN I. SPEIRS
Food Research Laboratories, Microbiology Division, Health and Welfare Canada, Ottawa, K1A OL2, Canada

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A lettuce floc was prepared and used for recovering enterovirus from an aqueous suspension. The method is simple, and the adsorption of coxsackievirus B5, echovirus 7, and poliovirus 1 is quantitative. The virus-floc complex may be removed from aqueous suspension by low-speed centrifugation and dissolved at an alkaline pH in a small volume of water; virus is then available for assay on cultured cells. Flocs from some other green vegetables also possess the property of virus adsorption.

A variety of adsorbents, both floccular (6, 7, 9; B. England, Bacteriol. Proc., p. 194, 1970) and granular (3, 5), have been used to recover virus from aqueous suspensions. Some are simple chemicals, e.g., aluminum hydroxide (9) or iron oxide (5); others are complex polyelectrolytes (2, 3, 8). The concentration of viruses by adsorbents is simple, fast, and economical. Although their scope may be limited to filtered or finished waters, they have been useful in recovering virus in a number of situations (2, 8-10).

The present report describes a simple method for recovering and concentrating virus adsorbed on a floc derived from plant tissue. In experiments on virus recovery from foods, it was observed that a floc, formed by acidifying a water extract of head lettuce, adsorbed coxsackievirus B5 from aqueous suspensions. We therefore investigated the efficiency of this adsorption for three types of enterovirus and examined several other vegetable extracts for this property.

MATERIALS AND METHODS

Viruses. Coxsackievirus type B5, obtained from Microbiological Associates, Inc., Bethesda, Md., was grown in HEp-2 cells. Echovirus type 7 and poliovirus type 1 (Sabin), obtained from the Virus Laboratories, Canadian Communicable Disease Centre, Ottawa, Canada, were grown in Vero and HEp-2 cells, respectively. Stocks were stored in sealed ampoules at −70°C. Dilutions were made in phosphate-buffered saline.

Cell cultures. HEp-2 cells, obtained from Microbiological Associates, Inc., and Vero cells, from the American Type Culture Collection, Rockville, Md., were grown in medium 199 containing 10% fetal bovine serum. Stock cultures were grown in Roux bottles as monolayers at 36°C. Monolayers for virus assay were prepared in 60 by 15 mm plastic dishes 24 h prior to assay and contained 3.0 by 106 cells at the time of use.

Virus assay. Monolayers were drained of fluid. Each dish received 2.5 ml of virus-containing sample. Cultures were shaken for 2 h on a rotary shaker at 120 rpm at ambient temperature (about 22°C) in a humidified CO2 atmosphere (5). Liquid was then removed and replaced with a 5-ml agar overlay containing 0.5% lactalbumin hydrolysate, 0.1% yeast extract, 10% fetal bovine serum, 0.15% sodium bicarbonate, and 1% agar (Difco) in Earle balanced salt solution. Monolayers were incubated at 36°C in a humidified CO2 atmosphere. Plaques were read after 3 days.

Lettuce floc preparation. A head of lettuce was quartered and blended at high speed in a Waring blender with water added equal to one-half the weight of the lettuce. The fine puree was centrifuged at 1,000 × g for 20 min, and the supernatant fluid was clarified by subsequent centrifugation at 15,000 × g for 15 min. The supernatant fluid (lettuce extract) was stored at −20°C in screw-capped bottles. A 426-g lettuce, homogenized with 213 ml of water, produced 310 ml of lettuce extract; the pH was 6.2.

The clear amber lettuce extract was a colloidal dispersion, as was demonstrated by its Tyndall effect. Floc formed when the pH was adjusted from 6.2 to 4.5 with 2 N HCl. Because lettuce floc settled rapidly, it was resuspended by pipetting before portions were removed for virus adsorption.

Assay of virus adsorbed by lettuce floc. An inoculum of 100 plaque-forming units (PFU) of coxsackievirus B5 in a 0.1-ml volume was added to 500-ml Erlenmeyer flasks containing 100 ml of distilled water. For the virus control, a similar inoculum was added to 5 ml of medium 199 containing 10% fetal bovine serum. Resuspended lettuce floc was added in volumes of 1, 2.5, 5, or 10 ml. No adjustment was required to maintain the pH at 4.5. The mixtures were shaken mechanically for 10, 30, or 60 min and centrifuged at 1,000 × g for 10 min to sediment the floc. The pellet was suspended in 4 ml of water and restored to the colloidal state by the addition of 1 N NaOH to a pH of 7.5. A 1-ml portion of a 1:1 mixture
of fetal bovine serum and 10 x medium 199 (pH 7) was added to each sample. Samples and virus control were assayed on two cell monolayers. Plaque counts on the duplicate dishes were added and expressed as percentage of the recovery from virus control.

Adsorption of echovirus 7 and poliovirus 1 was determined in the same way, but only 10-ml volumes of lettuce floc were used.

**Adsorption of virus by other vegetable flocs.** Extracts and flocs from the tops or heads of Boston lettuce, chicory, escarole, parsley, rappini, and Swiss chard were prepared as described for lettuce. A 10-ml quantity of each vegetable floc was added to 100 ml of water containing 100 PFU of coxsackievirus B5, and the mixtures were shaken for 30 min. After centrifugation, concentrated floc was dissolved at a pH of 7.5 to 8.5.

**RESULTS**

A linear relationship existed between the amount of lettuce floc and the efficiency of adsorption of 100 PFU of coxsackievirus B5 from 100 ml of water after a 10-min shake period (Fig. 1). All of the virus was recovered with 10 ml of lettuce floc, which represented 16 mg of floc by dry-weight determination.

With amounts of floc less than 10 ml, adsorption was not improved by increasing the shaking times from 10 to 30 or 60 min (Fig. 2).

A recovery of 85 and 90% of echovirus 7 and poliovirus 1, respectively, was achieved with 10 ml of lettuce floc from a 100-ml sample after a 10-min shaking period.

The extracts of six other vegetables produced floc at pH 4.5. Adsorption of coxsackievirus B5, ranging from 61 to 100%, occurred with all flocs except that from Swiss chard (Table 1).

**DISCUSSION**

Adsorption of enterovirus to lettuce floc is efficient, rapid, and simple. The forces that attract virus are probably electrostatic. This attraction is shared by five of six flocs prepared from other green leafy vegetables; only floc from Swiss chard had no adsorptive property.

A prominent feature of floc is its insolubility at acid pH and solubility (colloidal) at alkaline pH. In this respect, it differs from virus adsorbents described by other investigators. Virus adsorbed to polyelectrolytes, iron oxide, and alum must be eluted (at alkaline pH) before assay in cultured cells (5, 7, 8). Suspended, precipitated salts, however, such as aluminum phosphate, aluminum hydroxide, and calcium hydrogen phosphate, may be directly assayed on cells for adsorbed virus (9). Vegetable floc, with respect to solubility, resembles the alginate filter which is soluble in a 3.8% solution of sodium citrate (1). With both of these virus recovery methods, the final sample appears to be nontoxic to cultured cells.

Our most recent findings indicate that flocculation is not a prerequisite for adsorption; a colloidal lettuce extract (at a pH above 5.3) is also a powerful adsorbent not only for enterovirus, but also for reovirus and adenovirus. Vegetable flocs or colloids may be a useful and

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**Fig. 1.** Relation of volume of lettuce floc to adsorption of coxsackievirus B5 from 100 ml of water after a 10-min shaking period.

**Fig. 2.** Effect of time on the adsorption of coxsackievirus B5 from 100 ml of water with 1 (●) or 5 ml (▲) of lettuce floc.

**Table 1.** Recovery with vegetable floc of coxsackievirus B5 suspended in 100 ml of water

| Vegetable       | Input virus (%) |
|-----------------|-----------------|
| Boston lettuce  | 94              |
| Chicory         | 100             |
| Escarole        | 76              |
| Parsley         | 100             |
| Rappini         | 61              |
| Swiss chard     | 0               |
economical means of recovering viruses from finished waters.

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