

## THE SEARCH FOR ANTIVIRAL AGENTS AMONG CORYNEBACTERIA

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Received August 21, 1979; revised May 21, 1980

*Summary.* — Extracts of strains of *Corynebacterium diphtheriae* var. *mitis* avirulent, *C. hofmannii*, *C. xerosis*, *C. parvum* and an unidentified *Corynebacterium* species were tested for antiviral activity against laboratory strains of echovirus type 11, adenovirus type 2 and vaccinia, and a recent isolate of herpes simplex virus. Unlike a previous study, no appreciable antiviral activity was detected in the extracts tested.

*Key words:* antiviral agents; corynebacteria; echovirus; adenovirus; vaccinia virus; herpes simplex virus

It has been reported that extracts of certain members of the Corynebacteriaceae possess activity against a wide range of viruses (Carver and Naficy, 1962, 1964; Naficy *et al.*, 1972). Since several species of corynebacteria were readily available, we tried to repeat and extend some of these observations.

Procedures for the preparation and assay of extracts were essentially those outlined by Naficy *et al.* (1972). Modifications consisted of (a) culturing individual bacterial species in three 125 ml prescription bottles (instead of Roux bottles), each containing 100 ml of Harley's broth (instead of Martin broth), and resuspension of bacterial cells after centrifugation, into 20 ml of normal saline; (b) growing the unidentified yeast in 3-100 ml quantities of Sabouraud's broth in 125 ml prescription bottles; (c) extension of incubation period to four days for the slower growing *C. hofmannii*, *C. parvum* and the unidentified *Corynebacterium* species; and (d) an increase of the sonication period to 40 to 60 min, since preparations gram-stained after sonication for 20 min still showed a high proportion of intact bacterial cells when examined microscopically.

*Bacteria.* The following strains of corynebacteria were obtained from the Manchester University Collection of Bacteria: *C. diphtheriae* var. *mitis*, avirulent (MUCOB 6), *C. hofmannii* (MUCOB 90), *C. xerosis* (MUCOB 255) and an unidentified species of *Corynebacterium* (MUCOB 256). A strain of *C. parvum* was received from Prof. J. G. Collee, Dept. of Bacteriology, University of Edinburgh, Scotland. Also tried were contaminants occurring in the laboratory: *Bacillus* sp., an unidentified gram-negative bacillus and an unidentified yeast.

*Viruses.* Laboratory strains of echovirus type 11, adenovirus type 2 and vaccinia virus; and a recent isolate of herpes simplex virus were studied. These viruses were selected as being those against which Naficy *et al.* (1972) had shown the extracts to be active. A laboratory strain of adenovirus type 5 was also studied. For use, viruses were diluted to contain 100 TCID<sub>50</sub>.

*Cell cultures.* Primary human amnion cell cultures were prepared as described by Hayashi and LoGrippo (1963). HEP-2, HeLa and Vero continuous cultures were propagated serially by standard methods.

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Repeated attempts failed to confirm the previous findings by Naficy *et al.* (1972) of a significant antiviral effect against echovirus type 11 (and the other viruses studied) with extracts of strains of corynebacteria. Several variations were introduced in the procedures in attempts to demonstrate a response. These were: (a) using the extract diluted 1 : 2 and 1 : 10, instead of 1 : 40, (b) exposing cultures to diluted extracts six and 24 hr before the addition of virus, (c) mixing portions of virus and undiluted extract, then incubating at room temperature for 2 hr before inoculation on the cell sheet, and addition of maintenance medium after 1 hr of adsorption at 37 °C, and (d) adsorption of undiluted extract for 2 hr at 37 °C, after maintenance medium and virus were added.

Results indicate that strain characteristics, both of bacteria and viruses, may have been important in the original demonstration of antiviral activity. Unfortunately, an attempt to obtain the actual strain of echovirus type 11 and the actual strains of corynebacteria used in that initial study, was unsuccessful. Possibly also important were the cell culture systems employed, as Naficy *et al.* (1972) recorded significantly higher titres of extracts against echovirus type 11 in monkey kidney and monkey stable cell cultures, than with primary human amnion cultures. Furthermore, the possible effect of the culture medium (and the degree of aeration) in stimulating the production of antiviral substance should not be overlooked.

Our failure to confirm the results of Naficy *et al.* (1972) indicates that the production of substances active against the viruses we employed, is not an invariable property of corynebacteria; and that such substances are only produced and demonstrable under particular experimental conditions.

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